BIOLOGICAL CONTROL OF INTERNAL DECAY IN CREOSOTED DISTRIBUTION POLES

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ABSTRACT

The use of biological agents both in the form of the pure cultures of *Trichoderma polysporum*, *T. harzianum* and *Scytalidium* FY strain and a commercial product in which all three are included (Binab FYT pellets) to control *Lentinus lepideus* both in the laboratory and in the field was investigated.

Autecological studies with the three control organisms show that *L. lepideus* (identified as a primary decay fungus in creosoted poles from the literature and from studies of poles in Scotland) was overgrown and lysed or lysed from a distance by all control fungi when grown on the same agar plates. Other organisms resident in poles produced either a similar result or produced a stalemate/ neutral reaction.

When grown in situations which prohibited soluble metabolite contact, or when control organisms in wood blocks were killed and the blocks thoroughly leached, volatiles produced by *Trichoderma* spp. caused lysis of *L. lepideus* and residues of both *Trichoderma* spp. and *Scytalidium* FY strain were shown to inhibit growth and decay by *L. lepideus*. Both *Trichoderma* spp. and *Scytalidium* FY strain produced water soluble antibiotics effective against *L. lepideus*. Volatiles of *Trichoderma* were shown to be most effective against *L. lepideus* and *Fomes annosus* but not against other decay fungi which were tested.

Field studies undertaken over a three year period on more than 200 poles showed that *Trichoderma* was easily established in poles and that this was not limited by pole moisture contents. Nutrient transfer was not found to take place from soils to poles. Pole age, decay status and presence of previous remedial treatment did not seem to prohibit *Trichoderma* establishment which was shown to be present in over 90% of poles inoculated with FYT pellets. *Scytalidium* was never isolated from either pellets or poles.

Extensive sectioning of poles showed that the spread of *Trichoderma* within poles was variable and was linked to the quantity of other organisms resident in the poles. The

incidence of *L. lepideus* in poles was reduced by 45% by either prior or subsequent inoculation with Binab FYT pellets.

Microbiological and storage conditions of the commercial product were monitored during the project and the potential use of biological control as one other form of preservation which may protect creosoted wood in service as distribution poles is discussed.

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CHAPTER 1

INTRODUCTION AND GENERAL LITERATURE REVIEW

1.1 WOOD

1.1.1 The Importance of Wood

Since the establishment of the earliest civilisations, wood has been one of the most widely used natural resources, providing shelter, a convenient substrate for tool production and perhaps most importantly a fuel source (Jane, 1957). Wood is still a fuel source in many technically developing countries and this is a significant drain on forest reserves. In the technically developed world, wood and wood products are much used as raw materials in various industries including paper manufacture, the production of synthetic textiles, building construction, the production of decorative furnishings and as distribution and transmission poles. The physical structure of wood its toughness and strength qualities, its easy conversion for usage as a construction material and perhaps very importantly, a natural beauty lacking in many man made compounds, explain this sustained usage even in the face of alternative materials. The natural size limitation of wood for use as a construction material has been overcome by modern technology whereby planks of wood can be glued together to form structural units of predetermined strength in the laminated beam process, and the fire hazard of wooden structures has been reduced with modern

fire retardants.

The use of wood does however have disadvantages in that wood is liable to deteriorate due to environmental factors such as fire, mechanical wear, weather damage, chemical decomposition (Goldstein, 1973; Goldstein and Loos, 1973) and most importantly by attack from living macro and microorganisms (Cartwright and Findlay, 1958; Scheffer, 1973). When the biological decay factor can be eliminated, as can be seen from standing buildings in Scandinavia and Russia, wooden buildings may last for more than 1000 years in undecayed form (King, 1981).

1.1.2 Economic Importance of Forestry and Related Industry in Britain with particular reference to Scotland

The realisation that wood is an easily renewable, appreciating resource (mature trees being available within 50 years of planting in the United Kingdom compared with the thousands of years required for the production of oil, coal and other natural resources), has resulted in an improved climate of opinion regarding afforestation. This is compounded by the upsurge of wood prices illustrated in wood import figures (Richardson, 1981) which show that although timber imports into the United Kingdom decreased by 14% in 1980 the cost of the imports were reduced by only £33 million from a previous £2754 millions, a

relatively insignificant saving. Only 8% of our current needs are presently home produced although Richardson (1981) has pointed out that Britain has the facility to produce 25% of her needs with regard to forest products without any reduction in agricultural output or loss of scenic value of our countrysides.

Scotland has a most important part to play in forestry and related industries. Of the 1,722,000 hectares under cultivation in Britain (1,355,000 conifers, 367,000 broadleaves) a total of 820,000 hectares (781,000 conifers, 39,000 broadleaves) are situated in Scotland. This area represents approximately 9% of the total land area of Britain a figure which is much smaller than those found in most developed countries around the world (Table 1.1). Further evidence of the capacity to increase forest production in Britain is provided by the fact that in Scotland alone, the Forestry Commission has a total of 61,300 hectares awaiting planting. There is some evidence however (Anon, 1980) that an upsurge in the forestry industry is underway in Britain indicated by increased new planting and restocking of forestry land as well as the projected figures for future wood production.

Almost all distribution poles used by Electrical Boards in Britain consist of creosote treated Scots pine poles. The majority of these are imported and a considerable

Country	Total Area (million HA)	Percentage of total area		
		Forestry	Agriculture	Urban & other
Great Britain	22.8	9	77	14
Belgium	3.0	20	50	30
Denmark	4.2	12	69	19
France	54.9	25	67	8
West Germany	24.3	30	55	15
Ireland	6.9	4	70	26
Italy	29.4	27	59	14
Luxembourg	0.3	32	48 (est)	20
Netherlands	3.4	10	76	14
Norway	30.8	29	3	68
Sweden	41.1	64	8	28
Finland	30.5	74	10	16
USA	919.0	33	19	48
Canada	922.0	35	7	58
USSR	2140.0	43	28	29
Japan	36.8	67	15	18

Table 1.1 Land Use - International Comparisons

Note 1. Information for each country is based on the latest available published information.

2. Forestry areas include unproductive woodland.

3. Other land includes mountains, tundra, desert, etc.

national economic saving could be made by utilization of native timbers provided these had similar qualities to imported species. Sitka spruce (*Picea sitchensis* Karst) currently constitutes 38% of the planted wood in Forestry commission and private woodlands in Scotland (Anon, 1980). The strength properties and the fast growth rate together with the final tree form make spruce an ideal wood species for use as distribution poles. However it is almost impossible to adequately preserve using conventional creosoting processes. This failure is attributable to inherent structural features, which if they could be resolved would bring about important savings in trade balances.

1.1.3 Decomposition of Wood in Britain by Microorganisms

Wood can be attacked and destroyed by a wide range of microorganisms and several major works have described the variety and interactions between these organisms.

Fungal decay

The fungi are responsible for the greatest decay in wooden structures and their decay effects have been used as a means of categorising them into two groups, staining and mould fungi, and wood rotting fungi (Cartwright and Findlay, 1958).

The staining and mould fungi belong to the Ascomycetes and Fungi Imperfecti groupings and do not cause significant weight or strength losses in timber which they colonise. This is due to the fact that these organisms mainly invade the parenchyma ray cells which are a rich source of readily available nutrients (Corbett, 1963). Mould and staining fungi penetrate wood via intracellular bordered pits and occasionally bore holes are produced (Liese and Schmid, 1961; 1964; Sachs, Mair and Kuntz, 1967; Levy, 1967; Wilcox, 1973; Scheffer, 1973) which may result in increased radial permeability to preservatives (Chidester, 1936; 1942; Lindgren, 1952; Lindgren and Wright, 1954; De Freitas and Erickson, 1969).

The wood rotting fungi can be split into two groupings, soft rot fungi, which belong to either the Ascomycetes or Fungi Imperfecti, and Basidiomycetes.

The soft rot fungi produce a softening of the wood in its outer layers which led Savory (1954) to give them their name. They require high moisture levels and considerably higher protein contents than those present in wood to enable them to cause decay. They may also require a high substrate nitrogen content to enable them to manufacture the cellulase enzymes necessary for wood decomposition (Courtois, 1963; Liese, 1963; Corbett, 1965;

Schmid and Liese, 1965; and Levy, 1967).

The Basidiomycetes cause the most severe losses in the strength and durability of timber in countries with temperate climates and are able to decay wood even in environments with little moisture and utilizing only the proteins which are present. The Basidiomycetes are often classed as either "Brown rot" or "White rot" fungi depending on their ability to destroy the lignin layers of wood cells. The brown rot fungi by the use of cellulase enzymes remove the carbohydrate content of the wood while the white rot fungi sometimes referred to as "simultaneous rotters" have the capacity to produce both cellulases and ligninases and so utilize both the carbohydrate and lignin contents of wood cells (Liese, 1970; Scheffer, 1973).

Bacteria and Actinomycete decay

It has been considered in the past that bacteria required that wood be water laden before proliferation could take place, it is now accepted that the bacteria are among the earliest colonisers of wood even when relatively dry. However decay caused by bacteria is limited to wood with a relatively high moisture content and takes place over long time periods. In summary the decay effects produced by bacteria do not appear to be as important as those caused by the decay fungi although Liese (1970) has emphasised that there is still

much to learn about the role of bacteria in the biodeterioration of wood and synergistic activities such as nitrogen fixation may accelerate decay by other organisms.

The actinomycetes have only recently been implicated in the role of a wood decay organism (Harmsen and Vincents-Nissen, 1965; King, Eaton and Baecker, 1979; and Baecker and King, 1980) and it is obvious that further work in this area will be required before their role is totally understood.

Many environmental factors determine which class of organisms act on wood at any particular time, their effects on wood structure as well as determining the rates at which decay takes place. These include, moisture content, oxygen availability, temperature, pH, nutrient availability, geographic location, wood type, presence or absence of toxic materials and competition from other organisms. The susceptibility of wood to decay may therefore be seen to be a function of the interaction of a spectrum of organisms acting singly or together under changing environmental conditions on a wood substrate with an inherent variable decay susceptibility which may be influenced by the addition of toxic materials.

1.1.4 Preservation of Wood - with particular reference to Distribution Poles

In theory wood preservatives must protect timber throughout its required service life under a variety of exposure conditions. Thus preservatives used to protect marine poles must resist leaching, erosion by tides and marine organisms and those used to protect distribution poles and fence posts must be treated with systems which are effective in wood exposed to both air and soil.

The effectiveness of a wood preservative treatment depends on the expected performance of the timber structure, the efficiency of the treatment along with the toxic qualities of the preservative. The efficiency of the treatment ultimately depends on factors, such as penetration of preservative, permanence of preservative and the distribution within the wood to be protected.

Penetration of preservative even when pressure impregnated is controlled by a number of factors. These may include inherent permeability of the wood species, degree of pit aspiration, density, size of sample, resin content, moisture content, degree of mould infestation, earlywood-latewood ratio and sapwood depth.

Permanence of preservative is essential if the treated wood is to have any long term protection and unless the formulation of toxic chemicals are chemically complexed with the wood some degree of loss is inevitable.

Preservative distribution is relevant at two levels, preservative macrodistribution, i.e. the extent to which preservative is distributed between elements and micro-distribution, the extent to which preservative is distributed within one element e.g. it is thus possible to have good macrodistribution and bad micro-distribution as illustrated by some water borne preservatives.

Preservatives used to treat distribution poles in the United Kingdom may be for convenience categorised into two major groups.

Creosote oils

Coal tar creosote has been regarded as a standard preservative for over 100 years, Bethell's patent for the use of 'dead' oil of tar having been taken out in 1838. Creosote oil is obtained by the distillation of coal tar, it contains hydrocarbons, phenols and tar bases and distils above 205°C. It is a recognized preservative throughout the world and is used for most distribution and telegraph poles in Britain. The creosote is applied by pressure impregnation by one of three available

processes. Poles used by electrical authorities in Britain are impregnated using the Rueping empty cell process which consists of a cycle of operations as follows:-

a) Wood is dried to a moisture content of 25-30%.

b) Timber is placed in a pressurized cylinder and the air compressed for between 10-60 mins.

c) Preservative is pumped into the cylinder at temperatures of between 150-210° F whilst the air pressure is maintained.

d) Hydraulic pressure is then applied to force the hot preservative into the timber until the required absorption is achieved.

e) Pressure is released and the cylinder drained, during which time the compressed air expands.

f) A final vacuum is applied and the air that has been retained in the inner parts of the wood is permitted to escape. In doing so it forces out excess creosote, leaving the cell walls coated with creosote.

The Lowry pressure impregnation process varies slightly in that the initial air pressure is not compressed. The Bethell full cell process is similar except that a vacuum is drawn before the addition of the creosote thereby removing all the air and allowing the wood cells to be completely filled with creosote. The problem with creosote treatment is the large variability in the levels of uptake by different poles (Plate 1.1) which is sometimes associated with inadequate drying of stock prior to impregnation. This results in premature failure in the poorly protected samples.

Creosote is usually considered very effective in preventing external decay of poles by soft rot fungi possibly due to the sterilizing effect on the soil of the creosote which bleeds out of the pole and therefore not permitting the fungal contact between soil and wood necessary for soft rot action. Mowe, King and Senn (1983) have shown that some soft rot fungi actively grow away from creosote treated wood in response to volatiles emanating from the creosote. However during the study described in this thesis some soft rot cavities have been found in inadequately creosote treated poles (Plate 1.2).

The toxicity of creosote does not appear to decrease with age and Smith and Savory (1964) showed that aged creosote extracted from wood after 30 years of service

Plate 1.1 Variability of creosote treatment in Scots Pine poles.

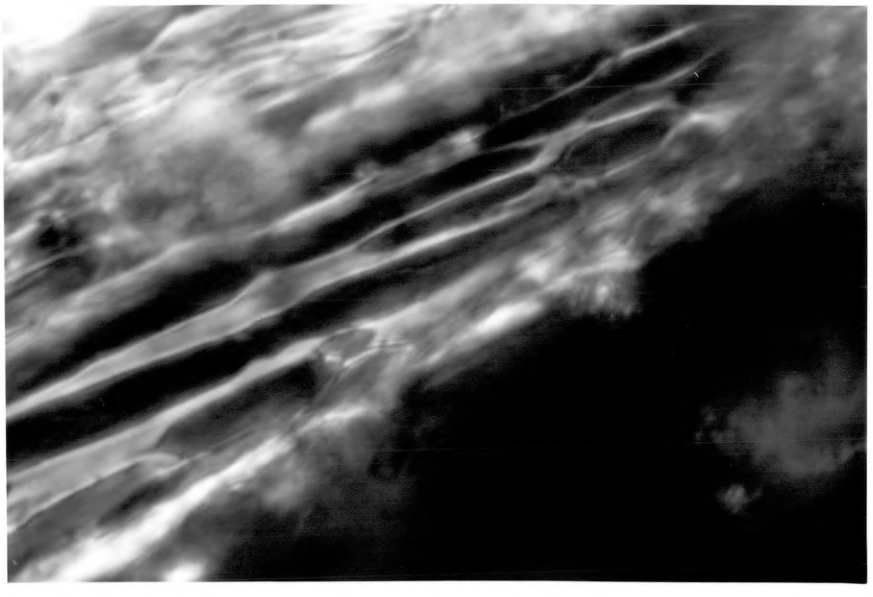


Plate 1.2 Soft rot cavities in fibres of creosote treated Scots Pine poles (x 1000) Polarised light illumination.

was found to be as toxic as new creosote to *Lentinus lepideus* when impregnated into wood blocks in the laboratory.

The major decay problem in creosote treated poles is caused by basidiomycetes which decay the region of untreated sapwood between the heartwood and the internal limits of creosote penetration. The fungus usually gains entry into the pole via cracks or bore holes. The basidiomycete most responsible for causing internal decay in creosoted distribution poles in Britain is *Lentinus lepideus* (Fr) which has the ability to tolerate relatively high levels of creosote (Cartwright and Findlay, 1958).

Waterborne Preservatives

The concept of a permanent preservative which could be easily applied using cheap solvents such as water has always been appealing. Among those most commonly available are Ammonical Copper Arsenate (ACA), Acid Copper Chromate (ACC), Chromated Copper Arsenate (CCA), Chromated Zinc Chloride (CZC) and Fluor-Chrome-Arsenate-Phenol (FCAP) (Hartford, 1973). Gray and Dickinson (1982) have recently proposed the use of Chromated Copper Arsenate Boron (CCAB).

CCA is the most widely used waterborne preservative which is also applied using vacuum impregnation. As with creosote, the timber should be seasoned to approximately 25% moisture content (B.W.P.A. Wood Preservation leaflet No. 10). As a full cell process is used drying periods of considerable lengths may be required after treatment depending on wood size. Timber thus treated can be painted easily and no odour emanates from the finished article.

Green timbers can also be treated with waterborne preservatives by either diffusion or sap displacement techniques. The diffusion process involves the spraying of green wood with the preservative and then leaving the timber to slowly dry for periods of 4 to 12 weeks to allow complete diffusion of the preservative into the wood. Sap displacement involves removal of the sap from the green wood while simultaneously replacing it with preservative. Although waterborne treatments have been used extensively in softwoods throughout European and Commonwealth countries and CCA treated poles are now more common in these countries (Britain excluded), problems exist with some hardwoods due to their higher susceptibility to attack by soft rot fungi.

Remedial treatments of creosoted materials

1. 'Cobra' process

This is the most widely used remedial treatment of creosoted distribution poles in Britain. The process involves approximately 40-50 separate injections of a flouro-arsenic based paste into individual poles over an area covering from one foot below ground to a height of three feet above. The poles are then surface treated with creosote before being wrapped with a protective metal sheet to deter animals from licking the poisonous salts. The salts migrate into the pole and completely sterilize the ground line area of the pole. Henningson and Nilsson (1975) however have shown that 9 years after treatment the microbial status of the poles had returned to their pretreatment state.

2. Toxic salt bandages

Toxic salt bandages have been used for the remedial treatment of creosoted poles (Koester and Weidler, 1956; Becker, 1957; 1960; Bavendamm, 1958; Becker and Zycha , 1958). This process involves wrapping around the groundline of the pole, bandages which have been impregnated with toxic salts which then diffuse into the pole and so sterilize this decay susceptible area.

3. Boric Acid Application

The possible use of boric acid as a remedial treatment to protect creosoted railway sleepers has also been investigated and Bechgaard, Borup, Henningson and Jermer (1980) have proposed a system in which measured doses of either boric acid or boric oxide (B_2O_3) placed in cartridges could be inserted into the timber. Boron compounds could then absorb water to form a solution of boric acid which could diffuse and protect the timber.

Bechgaard *et al* (1980) found that when boric acid was added to creosote treated wood the combined effect of the two preservatives against *L. lepideus* was greater than the effect of either individually.

4. Fumigation

Methods for remedial treatment of poles involving the injection of volatile fumigants such as Vapam (sodium methyl dithionate), methyl bromide, Voralex (methylisothiocyanate) and Chloropicrin into the hollow decayed area have been studied by Ricard, See and Bollen (1968), Graham (1973a) and Graham *et al* (1976). Such a system would need to be repeated, as residual effects of these treatments are uncertain and Graham (1973a) has suggested a five year treatment cycle for some fumigants.

1.2 BIOLOGICAL CONTROL

1.2.1 Biological Control - a definition

The term 'Biological control' has been defined in many ways by various workers. H.S. Smith (1948) defined biological control as "The suppression of a pest by means of the introduction, propagation and dissemination of the predators, the parasites, and the diseases by which it is attacked." Baker and Cook (1974) have suggested that "The reduction of inoculum density or disease-producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of the environment, host or antagonist, or by mass introduction of one or more antagonists." More recently Aidley (1976) has proposed that "It is sometimes possible to utilize natural enemies - predators, parasites or microbial pathogens - in controlling a pest" and biological insect pest suppression is defined by Coppel and Mertins (1977) as "The directed use of parasitoids, predators and pathogenic microorganisms to reduce and regulate insect pest populations to sub economic levels." All the above definitions have the common denominator that the control is achieved by man's manipulation. P. DeBach (1974) defined biological control as being part of the broader overall phenomenon of natural control.

Natural control being the regulation of populations of organisms by biotic and abiotic factors within more or less regular limits. From this it is clear that biological control occurs when man utilizes or manipulates a system which has been shown to work successfully in nature.

1.2.2 A Brief History of Biological Control

Although the popularity of biological control has recently escalated, the concepts of using biological agents to protect crops and animals is not a new one. Selection of resistant strains of crops is possibly the oldest practical application of biological control and this practice could possibly date back as early as 8-10,000 B.C. (Coppel and Mertins, 1977). These authors noted that early mans realisation that certain strains or varieties of crops performed better than others, were more resistant to disease or gave higher yields was probably the first usage of biological control. Similarly domestication of wild felines and their usage as biological control agents by the ancient Egyptians to suppress mice and rats in their granaries is another early practical application.

In the middle ages biological control was also used unknowingly by horticulturalists who reduced incidence of apple canker by soil amendment (Austin, 1657; Worlidge,

1678, in Corke and Risbeth, 1980). Austin suggested that wounds produced after removal of cankered branches should be treated with washes containing cow-dung and urine. What was not obvious to Austin was that the high nitrogen content of the wash favoured other competing organisms at the wound site to the detriment of the canker producing organisms.

Similarly Chinese citrus growers utilized the red ant (*Oecophylla smaragdina* F.) as a protective agent on their fruit trees and Groff and Howard (1924) noted that the large aggressive ants built their nests within the trees and drove off other insects which invaded their territory. By running bamboo pathways between the trees whole orchards were protected in this manner. Predation by ants has also been used in the Yemen in a similar manner to protect date palms (Clausen, 1978).

The utilization of biological control in agriculture as a major means of crop protection became a reality in the early 1880's. American citrus growers in California scientifically evaluated the possibility of crop protection from scale insects, mealybugs and other problem pest infestations by the introduction of natural enemies of these pests. This resulted in the importation of specific preditors to decrease pests in environments to which they are not native and perhaps the most successful illustration of this concerns the cottony-cushion scale

insect *Icerya purchansi* which is a pest of citrus fruit trees in California (De Bach, 1974).

A search for natural enemies of the scale was carried out in Australia and New Zealand the origin of the plant crop and it was found that a parasitic fly *Cryptochaetum iceryae* and a predatory ladybird *Vedalia cardinalis* were natural enemies which could be subsequently established in California. The result of importation of the Vedalia beetle was total elimination of the scale as a major problem within two years.

From the 1880's biological control has been used in increasing agricultural systems with varying degrees of success and more recently in the area of post harvest preservation.

1.2.3 Application of Biological Control in Agriculture

Problems encountered in agriculture can be classified into four areas, damage to crops by vermin, damage by insects, crop loss due to pathogenic microorganisms and suffocation of crops by weeds. Biological control can be implemented to ease the problems in each of these areas with varying levels of success.

1. Biological control of vermin

The introduction of the myxomatosis virus into wild rabbits remains the classic example of biologically controlling a mammal pest. It is probably the best known example of biological control at present in use. The success of the virus as a control agent is probably due to its acute host specificity, the virus is effective only against rabbits and even the closely related species of hare are not adversely affected (Vaughan and Vaughan, 1968). The disease is quickly and easily transmitted by a species of mite which are resident on the rabbits and so release of a few infected animals can result in quick and effective control.

2. Biological control of insects

Insect pests including aphids, mites, mealybugs, ants and some types of spiders as well as numerous species of caterpillars cause extensive financial losses by crop destruction. Biological control agents used to reduce insect numbers are very diverse including, insects, fungi, bacteria, viruses and even birds, amphibians and in rare cases mammals.

Insects are the most important biological control agents for bringing about the control of insects and may act either by parasitism or predation. In parasitism,

only one pest is controlled by individual control agents but with predation many pest insects can be devoured by one predator. However there are more parasitic insects used in biological control than predators and this probably relates to the specificity of host/ parasite relationships being more selective than predator/ prey relationships. Clausen (1956) reported that of 95 insects imported and established in the United States, 81 were parasitoids and De Bach's (1964,b) estimate showed that 18 of 24 introduced programs judged to be a success involved parasitoids.

Insect pests can also be controlled by microorganisms such as fungi and bacteria. *Verticillum*, *Hirstuella* and *Beauveria* control insects by parasitism whereby they grow throughout the thoracic cavity and absorb nutrients from the insects body before finally sporulating. Bacteria such as *Bacillus thuringensis* control by means of toxin release after ingestion by the insect with resulting paralysis and death. *B. thuringensis* is highly selective amongst the insect population and perhaps most importantly it does not exert any effect against bees which are essential for pollination purposes. The potential use of viruses for the control of insect pests has only recently been investigated but may become very important in the future. The virus acts by replicating within the cells of the pest producing the symptoms of viral disease and ultimately death.

The use of birds to control insect pests although of little significance compared to other control agents have been successfully introduced with reference to locust control by minah birds.

Insect populations can also be controlled by other related methods which include, cultural methods, crop rotation, irrigation and water management, better sanitation, genetic pest control, use of pheromones and competitive displacement of pests.

Genetic pest control involves manipulation of genetic material in a manner injurious to insect pests e.g. the 'sterile male' technique whereby male insects have genetic alteration so that dead offspring result from their matings. Pheromones have also been used to bait insecticide traps and so reduce pest numbers.

Competitive displacement of pest insects by non pests can be brought about by flooding the environment with large numbers of harmless organisms which are ecological homologs to the pest. There are many recorded cases of this type of control being used in the field (De Bach, 1966).

Some examples of biological control of insects and the mechanisms involved are shown in Table 1.2.

Type of Control Agent	Pest	Control Agent	Type of Control	Mechanism Applied by Biological Control Agent	Reference
INSECTS	Aphids	*Hippodamia* (common ladybird)	Predation	Simple predation	Van den Bosch and Hagen (1966)
	Cotton-cushiony scale	*Vedalia* beetle	Predation	Simple predation	Koebele (1890)
	Leafhoppers	*Geocoris* (Big eyed Bug)	Predation	Use of elongated proboscis for sucking body fluids from prey.	Van den Bosch and Hagen (1966)
	Aphids and fruit flies	*Braconids* (wasps)	External parasitism	External ovideposition with a venom which paralyses the host and so lessens the chance of it dislodging eggs.	De Bach (1974)
	Aphids and fruit flies	*Braconids*	Internal parasitism	Eggs laid through the body wall via a bore hole produced by specialised ovidepositor.	Van den Bosch and Hagan (1966)
FUNGI	Aphids & scale	*Verticillium lecani*	Parasitism	Spore contact results in germ tube growth into the host.	Hall (1980)
	Citrus rust mite	*Hirstuella thompsonii*	Parasitism	Invasive process unknown then total ramification	McCoy (1980)
BACTERIA	Widespread against lepidoptora	*Bacillus thuringensis*	Parasitism	Production of exo and endo toxins as well as ingestion of spores leading to lethal septicemia.	Dulmage (1980)
VIRUSES	Lepidoptora	Nuclear poly-hydrosis Virus	Parasitism	Virular replication within host cells with resultant cellular breakdown.	Ignoffo (1974)
BIRDS	Red Locust	Mynah bird (*acridotheres tristis*)	Predation	Simple predation	Greathead (1971)
AMPHIBIANS	Wide spectrum	Great toad (*Bufo marinis*)	Predation	Simple predation	Leonard (1933)
MAMMALS	Larchfly	Masked shrew (*Sorex cinereus*)	Predation	Simple predation	Warren (1971)

Table 1.2 Biological control of insect pests in Agriculture - some examples.

3. Biological control of disease producing microorganisms

Vascular wilts, cankers, silvering of fruit trees and rust and smut diseases of cereal crops are caused by pathogenic microorganisms including, bacteria, actinomycetes, viruses and various fungi. Successful control of most of these had been obtained by developing genetically resistant strains, however the ability of microorganisms to mutate and become effective against previously resistant strains has exposed some limitations in plant breeding as a total control mechanism. For example, different strains of *Fusarium oxysporum* Schlecht. f. sp. *vasinfectum Atk.* are now active against each of the cotton species, Upland, Egyptian and Indian originally bred for disease resistance against this fungus (Baker and Cook, 1974).

Biological control of pathogenic microorganisms by other microorganisms mainly involves the manipulation of either antagonistic or competitive strains so that natural interactions reduce or prevent plant disease (Cook, 1977).

When a competitive mechanism is involved the aim of the controller is to displace the pathogenic organism by swamping its particular habitat with excessive numbers of non pathogens. This practice can involve artificial inoculation of crops with immunizing commensals and was

first illustrated by Sandford (1926). He suggested that control of potato scab could be obtained by ploughing in green rye crops before potato sowing which results in bacterial proliferation which thus suppresses *Streptomyces scabies* in the soil.

When antagonism between commensal and pathogen is used to bring about control the actual mechanism involved can include predation, parasitism, the production of enzymes, antibiotics and volatiles. Much variation in particular mechanisms exist which may be specific to the antagonists involved as well as the microenvironment in which the interaction takes place. The importance of the environmental factors on control mechanisms is illustrated by the observation of Gregory *et al* (1952,b) who showed that some types of antibiotics (e.g. streptomycin) short lived in some soils, in others remained active for up to 14 days. Siminoff and Gottlieb (1951) concluded that the inactivity of some antibiotics around plant roots was attributable to their being adsorbed on to soil particles. The effect of temperature on the antagonistic effect of *Trichoderma viride* on *Fomes annosus* (Persson-Huppel, 1963) and the influence of acidity on antagonism between these organisms (Sierota, 1976) illustrates the effect the environment places on the use of fungi to control fungal pathogens.

Bacteria are probably the most important group of control agents as competitors for nutrients in the rhizosphere and around seeds. This is due to their ability to multiply rapidly in response to nutrients. They are immensely successful for protection of plants from species of *Fusarium* and other fungi which attack the plant roots by quick germ tube production at multiple points (Baker and Cook, 1974). The fact that some bacteria are anaerobic permits them to act as control agents in crop residues which have been ploughed under in wet conditions. The bacteria however do have limitations in that they are relatively inactive in dry soils. Bacteria do produce antibiotics although this is thought not to be a significant mechanism of the class with regard to biological control.

Actinomycetes owe their importance as biological control agents to their ability to produce antibiotics. Many actinomycetes have the ectoenzyme systems necessary to break down proteins, cellulose and chitin which increases their usefulness as control agents. Their ability to produce antibiotics however seem to be allied to a slow growth potential and this reduces their usefulness in biological control. They are therefore more useful against pathogens which take a while to become established rather than rapidly growing opportunistic pathogens.

Viruses can cause leakage of mycelial contents in some pathogenic fungi and they can be transferred via hyphal anastomosis. However there are no records of their use as a commercial biological control agent. Lemaire *et al* (1971) in Baker and Cook (1974) showed that infection of wheat with a viral infected strain of *Gaeumannomyces graminis* reduced further attack by an aggressive strain.

Although the fungi have received most attention from research workers, Baker and Cook (1974) suggested that they are probably of less importance in the protection of living plants from pathogens than the bacteria or actinomycetes. The soil saprophytic fungi however are the most important antagonists of pathogens during the breakdown of plant refuse. Fungi exert control mainly through antibiotic production rather than by competition although they may also exert control by volatile production. Fungi are used as seed dressings and are successful in this way against seed decay and seedling blight. Fungi may also act by direct predation and parasitism of plant pathogenic fungi by "hyphal interference", whereby the control fungi associates itself with the pathogen by intimate contact (coiling, encircling). This is followed by penetration and lysis of the pathogens mycelium and disintegration of the protoplasm due to the action of enzymes. An example of parasitism is the action of *Dactylella spermatophaga* on root rotting fungi whereby the

parasite produces hyphae capable of penetrating the oospores of the target fungi and so reducing their numbers (Drechsler, 1938). Many nematode species are plant parasites and so damage crops. They can also be controlled by fungi by various mechanisms such as snaring (as found in *Arthrobotrys oligiospora* Fres) or by *Dactyloria candida* (Nees Sacc.), via the ingestion or adhesion of spores which then germinate and permeate the body of the nematode. Some phycomycetes act by direct parasitism on nematode eggs in soil.

Some examples of biological protection of crops from pathogenic microorganisms are shown in Table 1.3.

4. Biological control of Weeds

The main agents of protection utilized for the control of weeds has been insects. They can be used to selectively consume a troublesome weed, however the problem is that if a single species of weed is to be controlled host specificity by the insect is of vital importance in order to protect surrounding plants from damage. Where control of a complex of plant species or total vegetation control is the goal little host specificity is required and it is in this area that biological control of weeds may be most useful. Plant pathogens have also been used to control weeds (Wilson, 1969) but when compared to insects they are of relatively minor importance.

Type of Control Agent	Pathogen	Control Agent	Type of Control	Method of Application	Reference
BACTERIA	*Fusarium roseum*	*Pseudomonadaceae*	Antibiotic production	Preinoculation of unrooted carnation cuttings before planting.	Michael and Nelson (1972)
	Xanthomonos transdicens sub sp *oryzicola*	*Erwinia* sp.	Not Ellucidated	Prespraying of crop with suspension of antagonist.	Rao, Devadath and Premlathadath (1978)
	Fusarium roseum	*Bacillus subtilis*	Competition for nutrients	Seed dressing with antagonist before sowing.	Chang and Kommedahl (1968)
ACTINO-MYCETES	*Fusarium oxysporum* and *Sclerotium rolfsii*	*Streptomyces gougeroli*	Antibiotic production	Addition of spore suspension to soil 15 days before sowing crop.	Mehrotra and Caludius (1972)
VIRUSES	Crown gall bacteria *Agrobacterum radiobacter*	Bacteriophage	Not Ellucidated	Absorption of bacteriophage by root immersion.	Boyd, Hildebrandt, and Allen (1971)
FUNGI	*Actinomyces scabies*	*Trichoderma lignorum.*	Antibiotic Production	Soil treatment and potato seed dressing	Daines (1937)
	Fomes annosus	*Peniophora gigantea*	Competition for nutrients	Spore suspensions are sprayed onto freshly cut tree stubs to prevent entry of pathogen.	Risbeth (1963)
	Fusarium roseum	*Mucor hiemalis*	Competition for nutrients	Soil treatment and seed dressings.	Lin and Cook (1979)
	Root rotting fungi	*Dactylella spermatophaga*	Parasitism	Artificial inoculation allows control fungi hyphae to penetrate oospores of pathogens.	Drechsler (1938)
	Stereum purpureum	*Trichoderma viride*	Undetermined	Inoculation of pruning wounds with spore suspension of control fungi.	Grosclaude, Ricard and Dubois (1973)

Table 1.3 Biological control of pathogenic microorganisms in Agriculture - some examples.

1.2.4 Advantages and Disadvantages of Biological Control

1.2.4.1 Advantages

Biocides have been used in agriculture since the middle of the eighteenth century due to their effectiveness in rapidly controlling pathogens. De Bach (1974) has pointed out the tendency for over utilization and that approximately 50% of insecticides currently used in agriculture are unnecessary. However, due to sales pressure many farmers consider them essential for protection of all crops. This has led to an increase in the numbers of biocide resistant pathogens and more than 200 species of insects, mites and ticks have developed resistance to one or more biocide (Baker and Cook, 1974). Very few species treated regularly with pesticides have not developed significant levels of resistance (Smith in Rabb and Guthrie, 1970). Under field conditions powdery mildews and other fungi have developed resistance to benomyl (Schroeder and Provvidenti, 1969); the wheat smut, *Tilletia foetida*, to hexachlorabenzene in Australia, and *Helminthosporium avenae* to organomercurials in Scotland, Ireland, Netherlands, New Zealand and the U.S.A. (Baker, 1972).

Over the past twenty years public awareness of pollution problems leading to potential ecological disasters associated with the wide usage of biocides has emphasised

a need for alternative control mechanisms which might include biological control. Van den Bosch (1978) considered biological control to be a total alternative to chemicals however it is more likely that integrated control between chemicals and biological agents seems a more realistic development.

Although many biological control systems have been commercialised others have failed not because the control achieved is unsatisfactory but because the cost of effecting control is greater than that of chemical pesticides currently used. Australian beetles *Cryptolaemus mantrouzieri* are reared on potato shoots before being released for protection of citrus trees in California from mealybug attack. Although this is a viable venture, when the beetles were first introduced to the United States they were reared on the citrus trees themselves which resulted in them being thought to be a financial failure as a control system. Most biological control systems, of predator/prey relationships type presently used, are commercially competitive in an economic market for biocides since once control has been achieved no further application of control agents are required unlike chemical biocides which must be applied on a regular basis.

Microorganisms are also clean and easy to apply either by sowing onto soil or spraying onto orchards. There are no recorded health hazards associated with their application unlike some chemical biocides which require stringent safety precautions to be observed during their handling. *Hirstuella thompsonii* used to control citrus rust mite is produced as a wettable powder composed of spores and mycelia which is simply sprayed over the trees at the required dosage to totally suppress mite populations.

Most biological control agents currently used are host specific. Most parasitoids but only some predators are monophagous, or slightly oligophagous, and this indicates a high degree of biological adaptation to hosts. There is also associated with this a rapid responsiveness to density changes in populations of target host (Coppel and Mertins, 1977). This is a common advantage of biological control over chemical treatments which frequently result in wide spectrum destruction of pests along with natural predators. This may result in population explosions of pests previously controlled by natural enemies. DDT, Carbonyl, Parathion, Eldrin and Dieldrin are wide spectrum general insecticides the use of which has resulted in explosive increases in populations of mites and scale insects previously not a problem on target crops (De Bach, 1974).

1.2.4.2 Disadvantages

Just as many environmentalists consider that usage of dangerous chemicals pollutes the environment with possible permanent effects, the usage of biological control might also upset the homeostatic balance of nature with harmful results. By utilizing biological control systems man is manipulating existing population dynamics in which some organisms with defined roles may be forced into extinction.

One of the major problems of biological control is the difficulty of obtaining a control agent capable of surviving in the absence of the pest without damaging the substrate and which can be effective at the time when the pest is most destructive. This involves the integration of three abilities by the control agent,

(a) Ecological compatability:- ability to function in the same habitat as the pest.

(b) Temporal synchronisation:- the assurance that no time periods are available when the pest is free from attack.

(c) Density responsiveness:- ability to increase numbers of control agents in response to pest population increase.

(Coppel and Mertins, 1977)

Many biological control agents shown to be effective under laboratory conditions fail when placed in the natural environment of the pest e.g. many fungi fail to act *in vivo* due to growth requirements of moisture, temperature or a lack of nutrients.

The term integrated control, a relatively new name for a long established practice, has come into general use to designate the combination of biological and chemical methods of pest control, whereby the maximum potential of each method is realized (Stern *et al*, 1959; Clausen, 1978). Problems arise when chemical control is used effectively for prevention of disease of crops on which biological control is to be used to eliminate further pests on the same or nearby crops. The Vedalia beetle had totally controlled scale insects on citrus trees in California between 1890 and 1946 until in 1946-47 DDT was widely used as a general insecticide on citrus trees resulting in elimination of the beetles. A population explosion of scale insects occurred producing major crop losses. However subsequent modification of chemical usage and reintroduction of the Vedalia beetles restored the previous control (Aidley, 1976). It is therefore important to ensure that biological control agents can live and function or are tolerant of chemical pesticides currently used in the environments to be protected.

A further problem which exists with the use of biological control is that control organisms are liable to mutation which may result in changes in the organism's ability to function as a control agent. Although this is unlikely to occur in insects it is quite possible when fungi, bacteria and viruses are used. Most importantly control agents may become pests due to such mutational changes. This is closely analogous to problems with chemical biocides whereby their effect is lost due to mutation in target organisms.

Furthermore the likelihood of hyperparasitism is also a problem, in which the controlling agents are susceptible to parasitism, disease or predation from their own natural enemies which numbers may increase significantly with the introduction of control agents. This problem may be overcome particularly with insects by screening potential beneficial organisms before their release into the environment in order to eliminate if possible any secondary parasitoids or predators which may be present (Coppel and Mertins, 1977).

1.2.5 Biological Control of Post Harvest Deterioration

To date little use has been made of biological control in prevention of post harvest deterioration largely due to problems involved in establishing commensals in substrates which will not be subsequently destroyed. Unless the

control agent acts by direct predation or parasitism on its host it must be equipped to utilize some portion of the substrate as a nutrient source without altering the structure or appearance of the product. An alternative is to incorporate a nutrient supply at the time of inoculation of the biological control system. Thus control of post harvest deterioration of crops, wood products, etc., is achieved by manipulation of either physical parameters, e.g. drying and storage under dry conditions or by chemical preservation.

Unlike most other natural substrates susceptible to post harvest deterioration, wood is not harvested for its food qualities but rather because of its structural attributes. When green, i.e. shortly after felling, wood behaves as a perishable raw material and is subject to microbial spoilage by a wide range of mould fungi which cause decolorisation and staining and by basidiomycetes which may rapidly produce decay. These phenomena are facilitated by the high moisture levels and the easy availability of soluble carbohydrates and nitrogenous materials present in wood sap.

It has been suggested (Bruehl, 1975) that possession of a substrate is "nine points of the law" with regard to competition for particular habitats and on this basis preinoculation of wood with commensal flora would be essential for control of decay fungi to be achieved.

Furthermore any commensal used should not possess the battery of enzymes required to breakdown structural components in wood in order that little damage be produced while the substrate was protected. Gillespie and Hulme (1970) have shown that hyphae of decay fungi spread rapidly through green wood establishing significant biomass before any discernable weight loss is produced and this growth is dependent on utilization of simple sugars. Hulme and Shields, (1970; 1972) showed that wood could be protected when green from decay fungi by prior inoculation with non-decay fungi which acted by competition for non structural carbohydrates present within wood. These authors used immunizing commensals of the non decay type including *Trichoderma viride*. Preinoculation with non decay fungi which consume the readily available simple sugars can inhibit biomass build up of decay fungi and thus produce biological control at least in the short term in green timbers.

1.2.5.1 Biological control of decay in Distribution Poles

Protection of dry wood poses a different series of problems to green wood protection in that it is much more difficult to establish the control organisms due to the lack of available nutrients in the substrate. An additional problem is that a wider range of basidiomycetes are capable of attacking the wood in the dry state. Due to these

problems little work has been undertaken to examine the potential of biological control to protect wood from decay.

The ability of the basidiomycete *Lentinus lepideus* to cause decay in creosoted timbers such as railway sleepers, distribution poles and mining timbers is well known (Cartwright and Findlay, 1958) and has been attributed to the high creosote tolerance of this fungus. Research for the Midlands Electricity Board in the United Kingdom (King and Penn , 1975, unpublished data) indicated *Lentinus lepideus* to be a major causative organism of internal decay in creosoted distribution poles in that region. Preliminary studies at this laboratory confirmed these results and showed that *L. lepideus* is the primary basidiomycete isolate associated with decayed poles in Scotland. Decay is normally initiated at the groundline and is usually confined in early stages to any untreated sapwood and contiguous heartwood within these regions of the poles. In Scotland decay by this organism is also found to occur at pole tops.

On detection of decay in poles (providing it is not too far advanced) various remedial treatments can be used to inhibit further development of decay fungi. Although most of these treatments show varying degrees of success all involve the use of potentially poisonous or dangerous chemicals which have been of increasing concern to

environmentalists. Biological control of decay fungi, using microbial antagonists, has been suggested as a desirable environmentally acceptable alternative to toxic chemical applications in certain situations provided that such antagonists could be shown to be as effective as conventional chemical preservatives. Ricard and Bollen (1968) first suggested the use of a *Scytalidium* sp. as a control agent for *Poria carbonica* Overholts in Douglas fir poles and these authors suggested that *Scytalidium* acted by producing a crystalline antibiotic with antibacterial and antifungal properties. The structure and activity of this antibiotic was later determined by Overeem and Mackor (1973) and Stillwell, Wall and Strunz (1973). Klingstrom and Johansson (1973) described antagonism by 38 *Scytalidium* isolates against decay fungi including *L. lepideus*. However Graham (1973) concluded that biological control of internal decay within Douglas fir poles using *Scytalidium* spp. was not effective due to the difficulties in establishing the *Scytalidium* in the poles and the high incidence of decay fungi in the poles 2½ years after inoculations.

Trichoderma species are presently the most utilized types of fungi in the field of biological control. They have been shown to be useful in both pre and post harvest situations and many varied mechanism have been postulated for their mode of action.

Ricard (1976) proposed the usage of species of *Scytalidium* and *Trichoderma* to control decay in standing creosote treated poles and preliminary studies at this laboratory have shown that both *Scytalidium* and *Trichoderma* are capable of overgrowing and causing lysis of *L. lepideus* mycelium when grown in agar culture. Oxley (1976) using a wood block system showed that both *Scytalidium lignicola* Pesante and *Trichoderma viride* Pers. ex S.F. Gray can control decay by *L. lepideus* and Morris and Dickinson (1981) have shown that *Scytalidium* FY strain induced death in *L. lepideus* mycelium when grown in competition in sawdust filled tubes. These workers evaluated the effects of actively growing antagonists against decay fungi under conditions highly conducive for growth. This would not be the case under field conditions as nutrient starvation might well result in early death of the non-decay producing biocontrol organisms unless supported by considerable nutrient supplement from sources external to the wood.

1.3 DESCRIPTION AND AIM OF PROJECT

The United Kingdom has a distribution pole population of 6 million(I.M. Fowlie, personal communication 1983), almost all of which are creosote treated prior to installation. The high cost of pole replacement (up to £ 250 per pole depending on size and location)

make it most important to increase the lifespan of each pole as much as possible. Various remedial treatments are available for this purpose however the 'cobra' process is currently the most widely used by some of the electrical authorities in the United Kingdom. Increasing pressure is being placed on the electricity boards by environmentalists who are concerned with the health and safety aspects of using potentially poisonous or dangerous chemicals. Radical alternatives, one of which is biological control, are therefore being sought to replace these chemical preservatives.

This thesis describes work undertaken as part of a CASE project sponsored by the North of Scotland Hydro Electric Board, the Midlands Electricity Board, Stokes Bomford (Chemicals) Ltd. and the Science and Engineering Research Council to evaluate biological control of *L. lepideus* in creosoted distribution poles in the United Kingdom. The biological control agent employed was the Binab FYT pellet, produced by Bio-Innovation Ab Binab a Swedish company. This pellet consists of a nutrient based bran mash incorporating spores and mycelial fragments of three fungi, *Trichoderma harzianum*, *Trichoderma polysporum* and *Scytalidium* FY strain.

The aims of the project are as follows:

1. To identify the basidiomycete organisms associated with groundline and pole top decay in interiors of creosoted poles.

2. To determine if growth of the commensal organisms could be produced within creosoted poles by preinoculation with FYT pellets.

3. To determine if creosote age, pole decay status and previous remedial treatments affected this colonisation process.

4. To monitor moisture and nutrient levels in on line poles and to evaluate their effects, if any, on establishment of the immunizing commensals.

5. To establish distribution of control agents after inoculation of poles and their interactions with *L. lepideus*.

6. To examine agar interactions of control agents, decay fungi and other resident organisms of distribution poles in service.

7. To determine if control of decay of wood could be achieved after death and leaching of the commensals.

8. To elucidate control mechanisms of *Trichoderma* against *L. lepideus*.

9. To advise the Electrical Boards as to the ability of the Binab product to combat internal decay of creosoted distribution poles.

CHAPTER 2

AGAR INTERACTION STUDIES

2.1 INTRODUCTION

The disadvantages of using agar studies to examine interactions between antagonistic microorganisms have been long recognised since it was first found that organisms antagonistic to pathogens in laboratory culture appeared to be ineffective in soil. Baker and Cook (1974) listed the more important reasons for this lack of transferability from laboratory to the field, particularly with reference to antibiotic production, as follows:

1. Laboratory studies usually involve only single cultures while field environments involve large populations.

2. Antibiotics may be produced on nutrient rich agar media but not in the nutrient deficient environments of the field.

3. Agar media tend to favour antagonists rather than pathogens.

4. Antagonists were free from competition on agar which may not be the case in field environments.

5. Pathogens may produce metabolites active against the antagonist which prevents the formation of antibiotics by them.

6. Environmental factors in laboratory studies are often far removed from those found in the natural environment.

7. Tests on agar tend to be more selective for antibiosis giving a distorted emphasis towards this type of antagonism.

8. Many pathogens remain as dormant spores in soils whereby they escape the effects of the antagonistic organisms while agar studies examine effects against actively growing pathogens.

However as long as field performance is monitored simultaneously, laboratory test methods can be a very useful first step in the search for biological control organisms. Large numbers of potential antagonists can be quickly and easily screened without the hardship and expense of testing every organism in a field system and furthermore, it is rare for a microorganism to prove antagonistic in soil but not on agar (Baker and Cook, 1974). However there are exceptions to this and the latter authors note that *Chaetomium cochlioides* is antagonistic to *Fusarium nivale* on oat straw but not on agar.

Due to their simplicity agar studies have been the forerunner to many biological control developments involving control of plant pathogens and wood decay fungi.

The most widely used method is that of simple direct antagonism between target and antagonist organisms achieved by inoculating the competitors at opposite sides of petri dishes filled with nutrient rich media suitable for the growth of both organisms (Weindling, 1932; Allen and Haenseler, 1935; Shields and Atwell, 1963; Ricard and Bollen, 1968; Chang and Kommedahl, 1968; Ricard, 1970; Klingstrom and Johansson, 1973; Grosclaude *et al*, 1974; and Morris and Dickinson, 1981). A similar technique involves the direct inoculation of the potential antagonist onto a previously grown lawn of the test organism (Ricard and Laird, 1968; Ishikawa *et al*, 1980). Growth of antagonists and test fungi on hanging agar smears (Aytoun, 1952) and on agar covered glass slides (Liu and Baker, 1980) are essentially developments of the same technique on a microscale to facilitate easier micrography of interactions. Some workers view cultural studies purely from the viewpoint of antibiosis between antagonist and target fungi and some simple techniques are used to monitor these reactions. These have included growing the antagonist on top of cellophane membranes placed over lawns of the target fungi and thus examining soluble antibiotic production (Lundborg and Unestam, 1980; Ishikawa *et al*, 1980), testing the effects of ether extracts of filtrates from antagonists upon growing lawns of the target fungi (Komatsu *et al*, 1969) and monitoring the growth of target fungi on sterilized filtrates from cultures of potential antagonists (Daines, 1937).

Other laboratory methods not involving agar have been devised to examine interactions between fungi and bacteria found in wood. Leben (1978) used tree branch discs as a medium on which to monitor interactions between wood decay pathogens and other potential antagonists. Morris and Dickinson (1981) used sawdust filled tubes which were inoculated at either end with test organisms. Although both these methods have their advantages in particular areas of study, agar interactions, due to their ease of application, are more commonly used as a first stage in the development of a biological control system.

During interactions on agar between antagonist and target fungi many mechanisms can be involved in bringing about the reactions which occur. These can include the production of antibiotics or soluble toxic metabolites, direct hyphal interaction between microorganisms, production of antimicrobial volatiles and competition for available nutrients. The latter mechanism is usually minimised in agar studies due to the abundance of nutrients present in the medium whereas it is often the most important mechanism during interactions in field situations in which nutrients are scarce. Further mechanisms which may occur in plants e.g. increase in the host plants' defence mechanism due to the action of antagonists which produce detrimental effects on potential pathogens, cannot be monitored from laboratory studies.

Rayner and Todd (1979) carried out extensive studies of fungi both in wood and on agar. Their findings show that in wood interactions between fungi can be classified into three categories,

(a) intermingling, whereby two fungi grow within the bounds of each others colony without adverse effect on either fungus.

(b) deadlock, when one fungal type stops growth at the boundary of another fungal colony.

(c) replacement, when one fungus replaces another in a particular area of the wood.

Using a direct antagonism test by means of cross plating on agar Rayner and Todd (1979) found that reactions between decay and non-decay fungi in agar "epitomize the range of interactional phenomena likely to be found in wood". The interactional phenomena observed during these agar studies may be described as follows.

1. Merging of mycelia between identical strains.

2. Growth of one fungus into or over another (replacement).

3. Deadlock, in which neither fungus was able to grow past the other.

4. Formation of coloured contact zones in the medium.

5. Lysis of one mycelium by the other, usually prior to or accompanying replacement.

6. Development of dense, sometimes coloured zones of mycelium in the region of contact between colonies.

7. Stimulation of fruiting.

8. Development of clear zones between colonies.

9. Production of leathery crusts of mycelium between colonies.

The decay of creosoted wood by *L. lepideus* occurs in an environment comparatively free of competing organisms and the use of agar studies as a first step to examine biological control of this fungus seems particularly appropriate.

Little work has been undertaken to examine agar interactions between biological control organisms and *L. lepideus*.

The experiments described in this chapter were undertaken to examine interactive effects between *Trichoderma* (isolated from Binab FYT pellets), *Scytalidium* FY strain and two strains of the decay fungus *Lentinus lepideus*. As the project developed a number of fungi resident in creosoted transmission poles were consistently isolated

and these too were examined for interactive effects. Because of the results produced from these studies other wood decay basidiomycetes were included in the study for comparative purposes.

2.2 MATERIALS AND METHODS

2,2,1 Test Organisms

The following test organisms were used in this study:-

Control fungi - A *Trichoderma* species isolated from Binab FYT pellets (hereafter referred to as Binab *Trichoderma*), *Scytalidium* FY strain and two pole isolates of *Scytalidium*.

Pole resident Organisms - *Fusarium* sp. and *Cladosporium resinae*.

Basidiomycetes - *Lentinus lepideus* (FPRL 7B, FPRL 7F), *Fomes annosus* (Fr.) Cooke (FPRL 41E), *Serpula lacrimans* (Wulf ex Fr.) Schroeter (FPRL 12C), *Coriolus versicolor* (Link ex Fr.) Quelet (FPRL 28A) and *Coniophora puteana* (Sch. ex Fr.) Koreten (FPRL 11E).

All control fungi were tested against *L. lepideus* 7F, 7B and against *Fusarium* and *Cladosporium* the two most regularly isolated resident pole organisms.

Binab *Trichoderma* was not only tested against *L. lepideus* but also the four other common wood decay basidiomycetes *C. puteana*, *C. versicolor*, *S. lacrimans* and *F. annosus*.

The two pole resident fungi were tested against the two strains of *L. lepideus* and also against each other to monitor possible effects of these fungi on the establishment of the decay fungus in creosoted poles.

Finally interactions were monitored between each of the four control fungi included in the study to examine possible combinations of biological control fungi.

Table 2.1 shows a summary of the interactive tests carried out during the work undertaken in this chapter.

2.2.2 Test Methods

Eight mm agar cores were removed from the margins of actively growing cultures of both target and antagonist fungi and placed, approximately 7 cms apart, at opposite sides of petri dishes containing 20 mls of 3% malt extract agar. Preliminary studies showed that all the organisms involved in this study had widely varying growth rates with those of *Trichoderma* and one of the *Scytalidium* pole isolates being particularly vigorous. Due to these differences, in almost every interactive test

Trichoderma	vs	*L. lepideus* (FPRL 7F)
"		*L. lepideus* (FPRL 7B)
"		*F. annosus*
"		*S. lacrimans*
"		*C. versicolor*
"		*C. puteana*
"		*Scytalidium* FY strain
"		*Scytalidium* (isolate 1)
"		*Scytalidium* (isolate 2)
"		*Fusarium* sp.
"		*Cladosporium resinae*
Scytalidium FY strain	vs	*L. lepideus* (FPRL 7F)
"		*L. lepideus* (FPRL 7B)
"		*Scytalidium* (isolate 1)
"		*Scytalidium* (isolate 2)
"		*Fusarium* sp.
"		*Cladosporium resinae*
Scytalidium (isolate 1)	vs	*L. lepideus* (FPRL 7F)
"		*L. lepideus* (FPRL 7B)
"		*Scytalidium* (isolate 2)
"		*Fusarium* sp.
"		*Cladosporium resinae*
Scytalidium (isolate 2)	vs	*L. lepideus* (FPRL 7F)
"		*L. lepideus* (FPRL 7B)
"		*Fusarium* sp.
"		*Cladosporium resinae*
Fusarium sp	vs	*L. lepideus* (FPRL 7F)
"		*L. lepideus* (FPRL 7B)
"		*Cladosporium resinae*
Cladosporium resinae	vs	*L. lepideus* (FPRL 7F)
"		*L. lepideus* (FPRL 7B)
L. lepideus (FPRL 7F)		*L. lepideus* (FPRL 7B)

Table 2.1 List of interactive tests involving control fungi, resident pole organisms or basidiomycetes undertaken.

one fungus was inoculated up to two weeks before the other to ensure that the estimated line of contact between the two colonies lay approximately in the centre of the plate.

Five replicate plates were set up for each cross and all plates except those incorporating *S. lacrimans* were incubated in the dark at 25 °C. Crosses involving *S. lacrimans* were incubated at 22 °C to facilitate the growth of that decay fungus.

Preliminary studies showed that *L. lepideus* was overgrown and lysed by *Trichoderma* with accompanying reddening of the autolysing mycelium of the decay fungus. Cores of reddened *L. lepideus* were removed from such plates and transferred onto 4 ppm benomyl agar (selective for basidiomycetes), to determine if regrowth could take place. If this did not occur it was then presumed that the decay fungus had been killed. Slides were prepared from this reddened mycelium to determine if morphological changes had taken place in these regions.

Using the criterion of reddened mycelium as a measure of extent of viable growth, the viable hyphal extension of both test and antagonist fungi was measured daily in the direction of the competitor.

Using the above technique interactive study plates between the two strains of *L. lepideus* and either Binab *Trichoderma* or *Scytalidium* FY strain were also set up at temperatures of 5 and 10°C.

One final interactive test was carried out in which a 'wild' strain of *L. lepideus*, isolated from a rotting pole top in Scotland, was inoculated onto a plate which was then seeded with three Binab FYT pellets.

2.3 RESULTS

A total of 32 cross reactions were carried out and although many different reactional phenomena were witnessed it was found that all the reactions could be classified into three broad reaction types. These were described as follows: Replacement, Intermingling or Stalemate.

1. Replacement reactions

This type of reaction was classified as replacement since it involved the replacement of one fungus in an area previously occupied by another to the complete exclusion of the previous occupant.

When Binab *Trichoderma*, *Scytalidium* FY or one of the two pole isolates of *Scytalidium* were tested for interaction

with either of the two strains of *L. lepideus* the outcome was always the same. The organisms grew until they made contact at the centres of the plates approximately, after which the *L. lepideus* mycelium started to turn red with accompany release of red pigment into the agar (Plate 2.1). *L. lepideus* was then overgrown by the antagonist organisms with increased reddening and pigment release as the antagonist front advanced over the *L. lepideus*. Figure 2.1 (A-M) shows how the extension of viable mycelia, of both antagonist and target fungi, varies with time during replacement type reactions.

On some plates mycelium of *L. lepideus* started to turn red and release pigment when the growing fronts of antagonists were still at a distance of 10 to 20 mm from the target fungi. Cores of such reddened mycelia removed from antagonism plates when tested on benomyl agar as previously described showed no subsequent growth proving that the decay fungus had been killed by the antagonist. Squash slides prepared from such reddened mycelium were examined and showed that cytoplasmic aggregation and hyphal cell wall lysis had taken place (Plate 2.2).

During antagonism between *Scytalidium* FY strain and *L. lepideus* a dense zone of fluffy *Scytalidium* mycelium formed along the line of contact between the fungi before the *Scytalidium* advanced over the decay fungus.

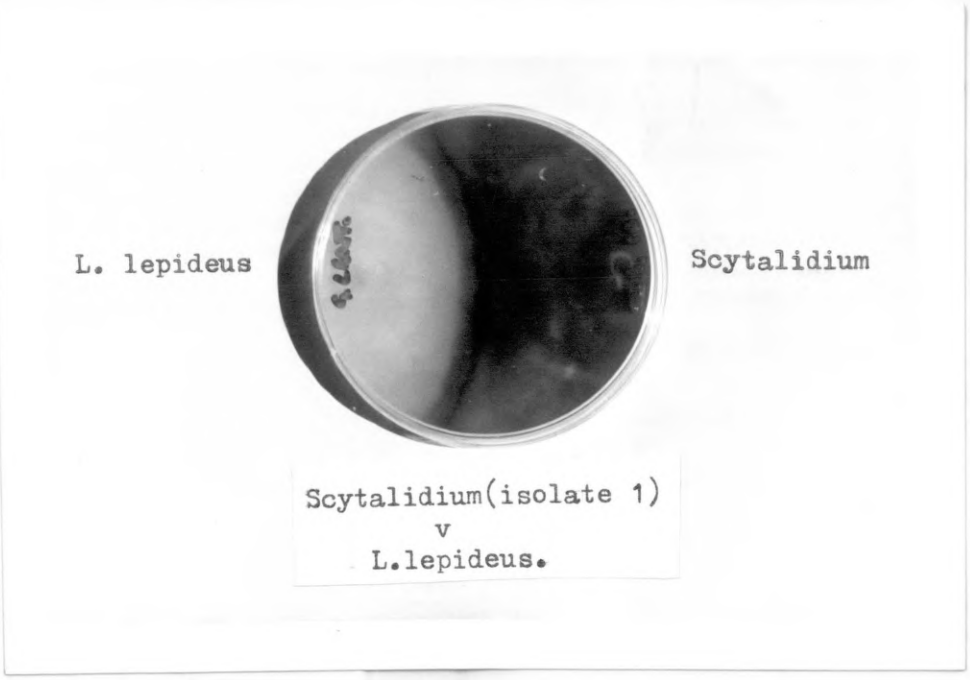


Plate 2.1 Reverse of Interactive test between *Scytalidium* (isolate 1) and *L. lepideus* showing discolouration of *L. lepideus* mycelium along line of contact between the two fungi resulting in a replacement reaction.

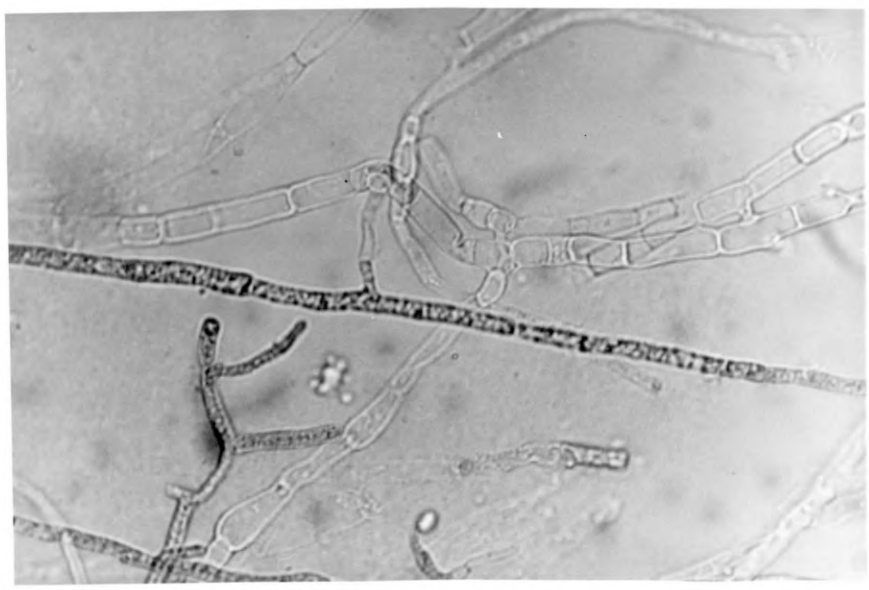


Plate 2.2 Photomicrograph of slide prepared from region of contact between *Scytalidium* FY strain and *L. lepideus* showing granular appearance of hyphae of the decay fungus while hyphae of *Scytalidium* remain unaltered.

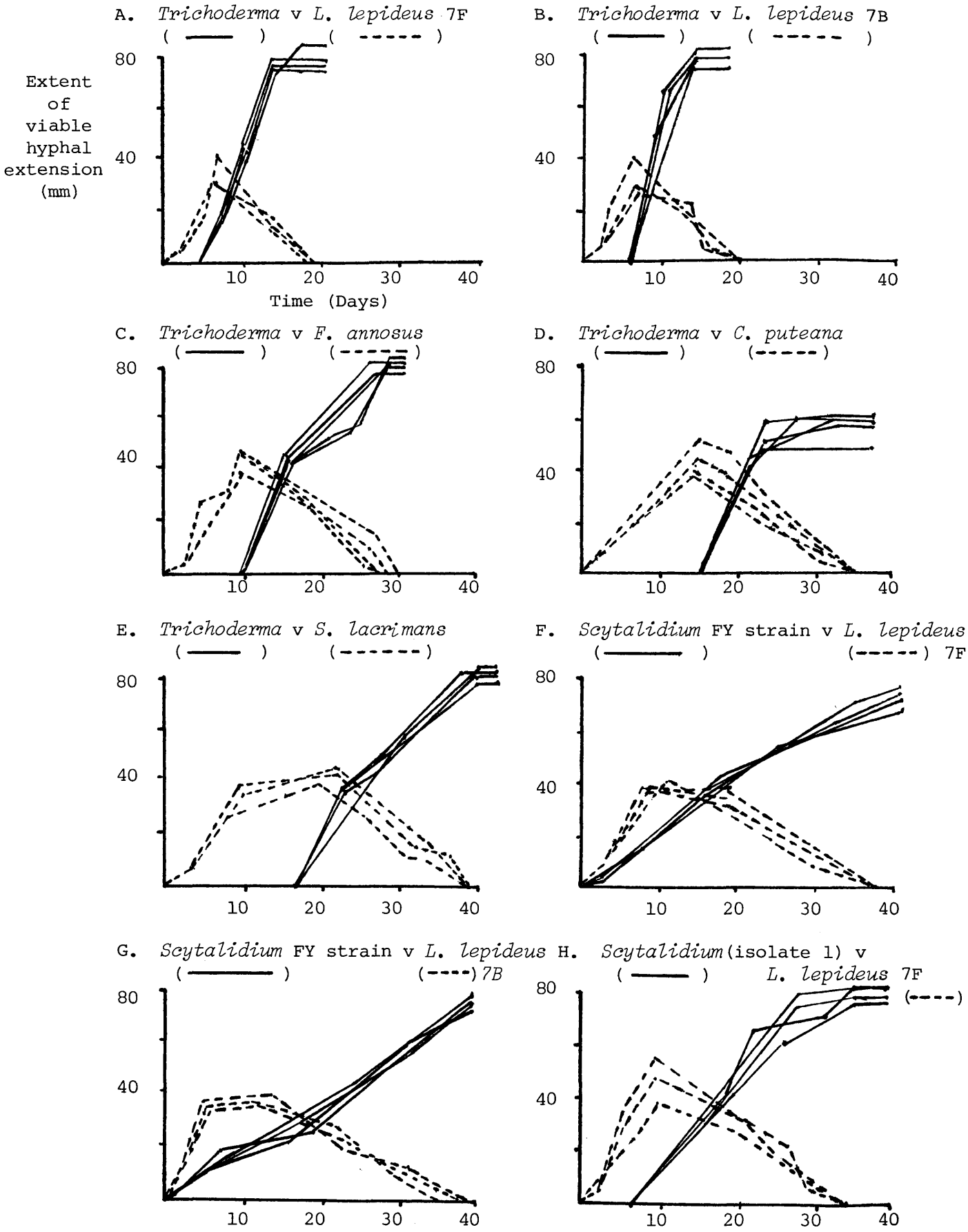


Figure 2.1 Interactions between control fungi, resident pole organisms and basidiomycetes resulting in replacement reactions, carried out at 25°C.

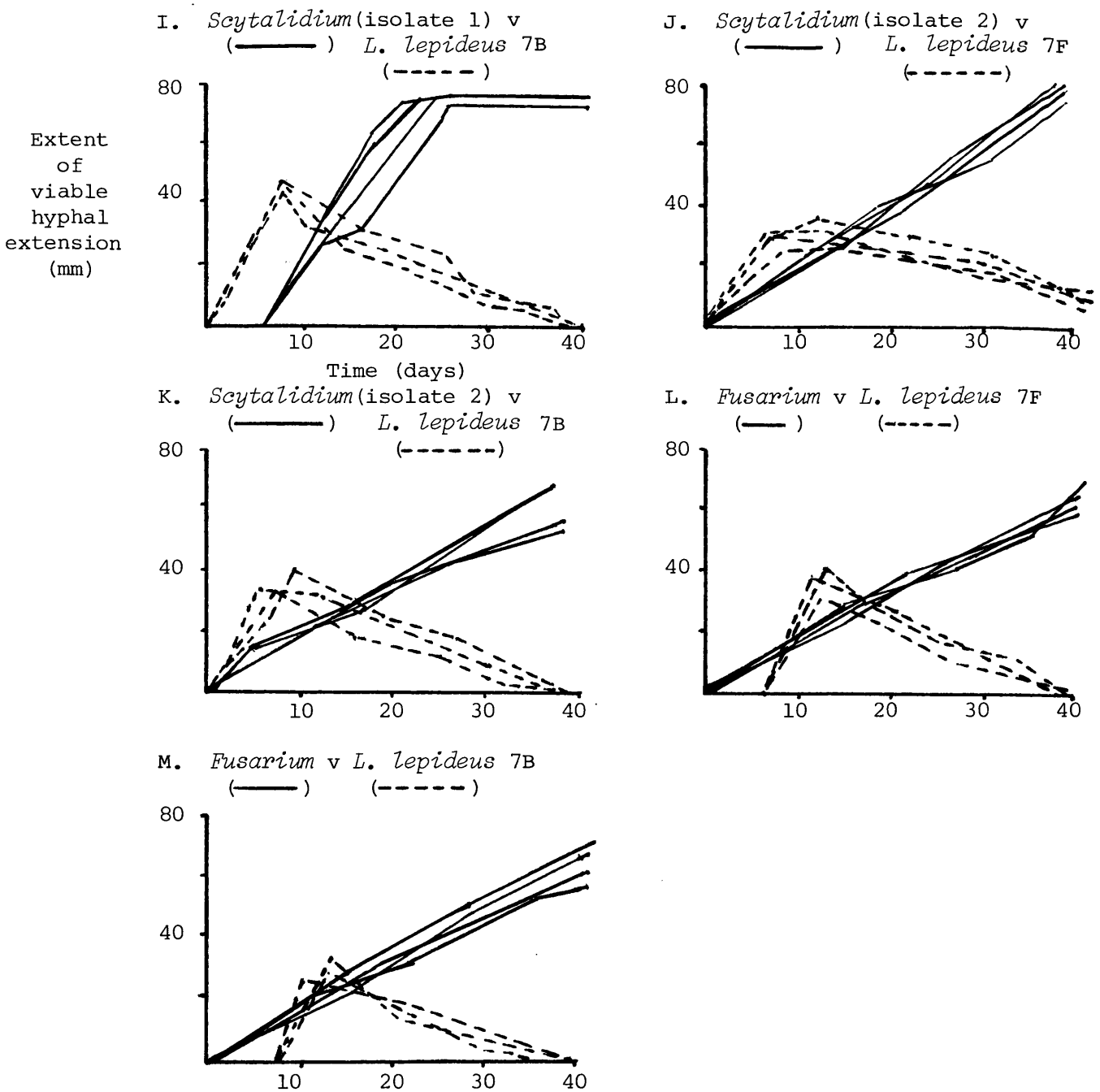


Figure 2.1 Contd.

When *Fusarium* isolated from the interior of a creosoted pole was tested against either of the two *L. lepideus* strains a similar reaction to that mentioned above resulted, with lysis and death of the decay fungus and accompanying pigment release. However the pigment release was never observed until contact had taken place between the two fungi.

When Binab *Trichoderma* was tested against either, *S. lacrimans*, *C. puteana* or *F. annosus* the results were identical to those found with the two strains of *L. lepideus*, complete replacement with accompanying overgrowth and lysis of the basidiomycete (Plate 2.3). However when Binab *Trichoderma* was tested against *C. versicolor*, it was unable to overgrow the decay fungus and a state of stalemate resulted.

Interactions between the two strains of *L. lepideus* and either Binab *Trichoderma* or *Scytalidium* FY were also carried out at 5 α 10°C in order to examine the antagonistic potential of these organisms at temperatures more closely related to the field environment. The results of these interactions at 10°C were the same as those found at the higher temperature although the growth rates of the two organisms were slightly reduced. At 5°C growth rates of the organisms were so slow that the agar plates had dried out before any significant contact of the fungi had taken place.

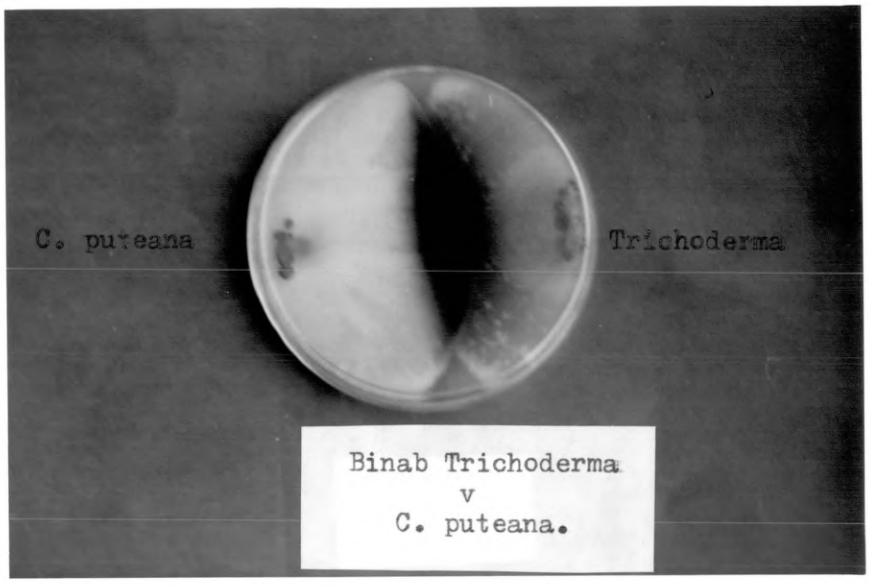


Plate 2.3 Reverse of interactive test plate showing replacement of *C. puteana* by Binab *Trichoderma* with accompanying reddening of the mycelium of the basidiomycete.

A supplementary experiment was undertaken in which FYT pellets were inoculated onto malt extract plates opposite a core of *Lentinus lepideus* which was grown up from wood isolated from a rotting pole in Scotland. The results were the same as those recorded using laboratory strains of *Lentinus* i.e. the decay fungus was overgrown and lysed by the advancing *Trichoderma* mycelium which eventually covered the whole plate. One interesting phenomenon found in these plates which was not witnessed in the other tests was the fact that once *Trichoderma* had overgrown the *Lentinus* colony and had itself sporulated, the spores produced above the dead *Lentinus* mycelium were yellow in colour compared with the green spores found over the rest of the plate (Plate 2.4).

2. Intermingling Reactions

When *Trichoderma* isolated from Binab FYT pellets was cross plated against *Scytalidium* FY or any of the resident fungi isolated from creosoted poles (i.e. *Fusarium*, *Cladosporium* and *Scytalidium* isolates 1 and 2) the outcome of the cross was always the same. The organisms grew out until they made contact at approximately the centre of the plate at which point the *Trichoderma* continued to grow over the other fungi which appeared to stop growing (Plate 2.5).

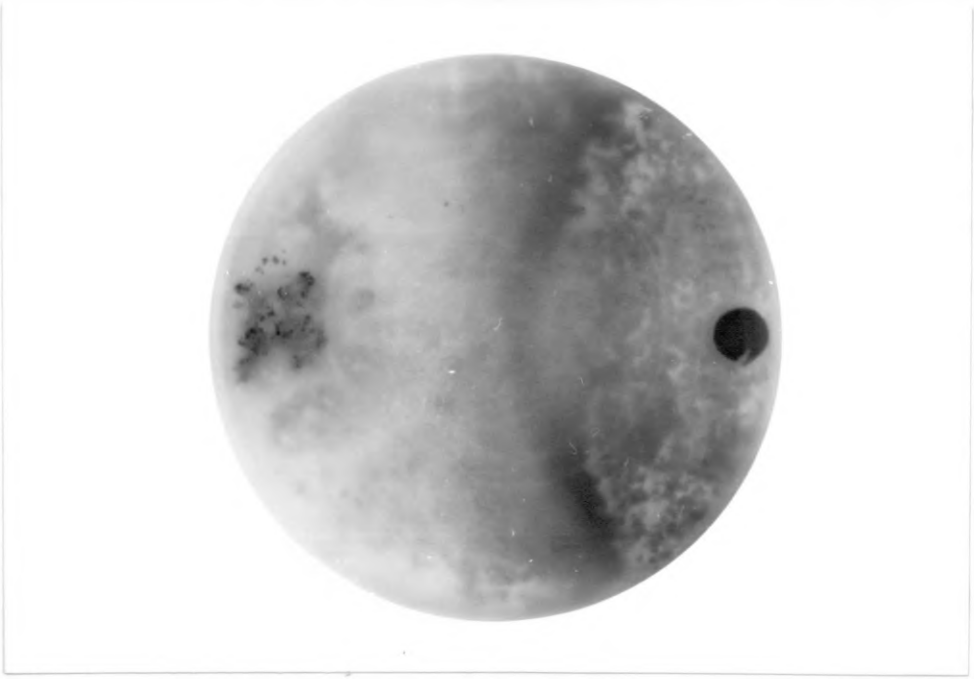


Plate 2.4 Interactive test plate showing replacement of *L. lepideus* by Binab Trichoderma.

Top - reverse of plate showing pigment release by colonised decay fungus.
Bottom - Effect on *Trichoderma* spore colouration during colonisation of *L. lepideus*.

The *Trichoderma* seemed to cause a state of fungistasis, although there was no evidence of death or lysis in the overgrown fungi which appeared to remain viable. Slides were prepared from the area of contact between the fungi however these showed no evidence of hyphal interaction or lysis of mycelium of either organism.

An identical reaction was recorded when pole isolates of *Cladosporium resinae* and *Fusarium* sp. were cross plated against one of the pole isolates of *Scytalidium*, the former fungi being overgrown by the *Scytalidium*.

This type of reaction can be classed as intermingling since it involves overgrowth of one fungi by another without any apparent lethal effect on either fungi although a state of fungistasis appears to be induced upon the overgrown fungus. Figure 2.2 (A-G) shows how the extension of viable mycelia of the two competitors varies with time during this type of antagonistic reaction.

3. Stalemate Reactions

When interspecies antagonist plates were set up or when *Cladosporium resinae* (the most common natural inhabitant of creosoted poles) was cross plated against either, *Fusarium*, *L. lepideus* (FPRL 7F, 7B) or two of the three *Scytalidium* species the outcome of the test was always the same. Both test and antagonist fungi grew out

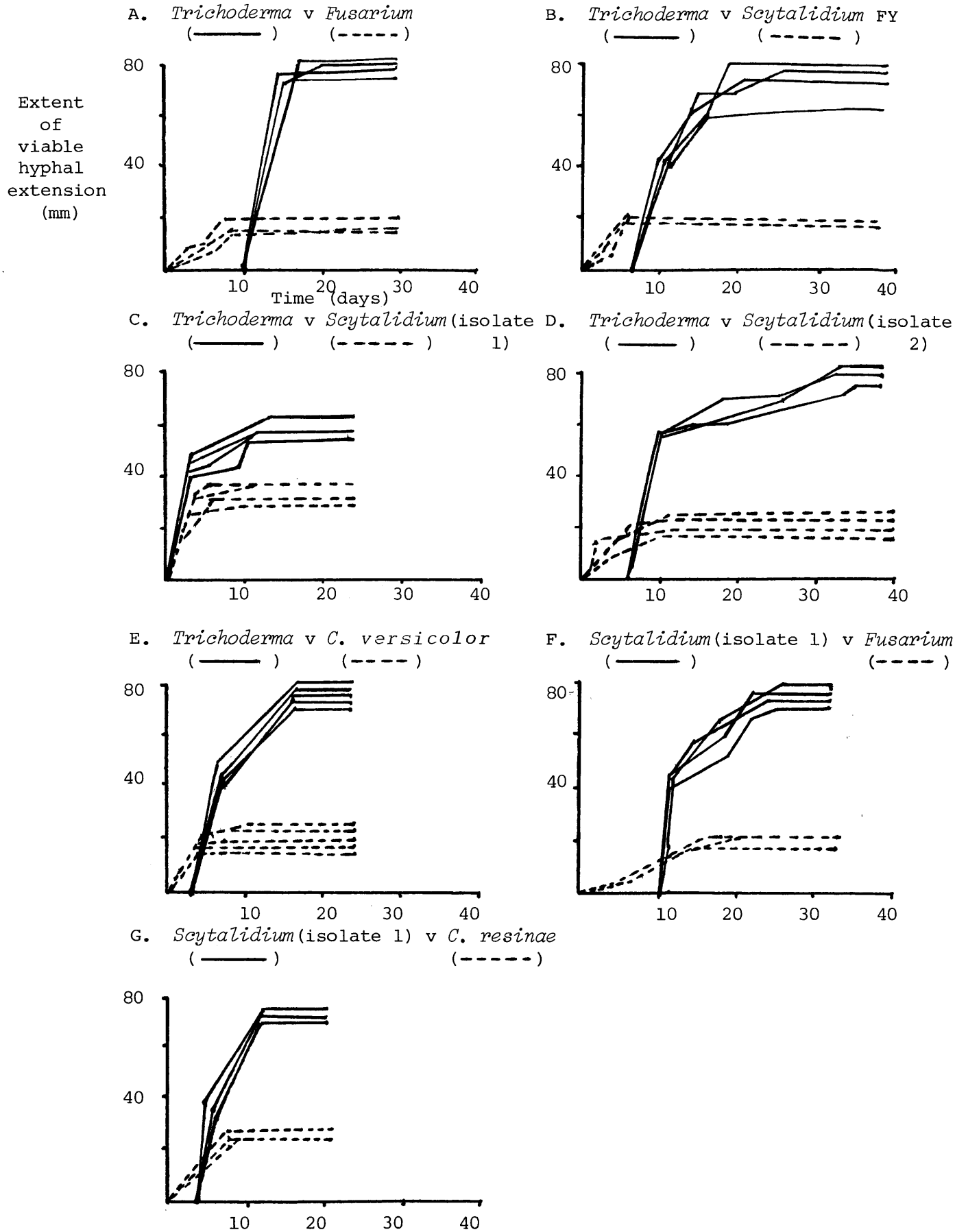


Figure 2.2 Interactions between control fungi, resident pole organisms and basidiomycetes resulting in intermingling reactions, carried out at 25°C.

towards each other until the point of contact was reached whereby a state of impasse occurred. Neither of the fungi appeared capable of overgrowing the other and microscopic examination of mycelium from the contact zone showed no hyphal interaction. A similar reaction occurred when the pole isolate of *Fusarium* was tested against either *Scytalidium* FY strain or one of the pole isolate *Scytalidium* species.

This type of reaction can be classed as stalemate since it involves no overgrowth or intermingling of hyphae by either fungus and no detrimental effects are produced (Plate 2.6).

Figure 2.3 (A-L) shows how the extension of viable mycelia of the two competitors varies with time during this type of antagonistic reaction.

Table 2.2 shows the resultant reaction types of all of the tests carried out during this study.

2.4 DISCUSSION

Interactional phenomena such as deadlock, lysis of mycelium accompanying replacement, development of dense zones of aerial mycelium, pigment release, changes in sporulation patterns and overgrowth of one fungus by another are similar to those observed during agar studies

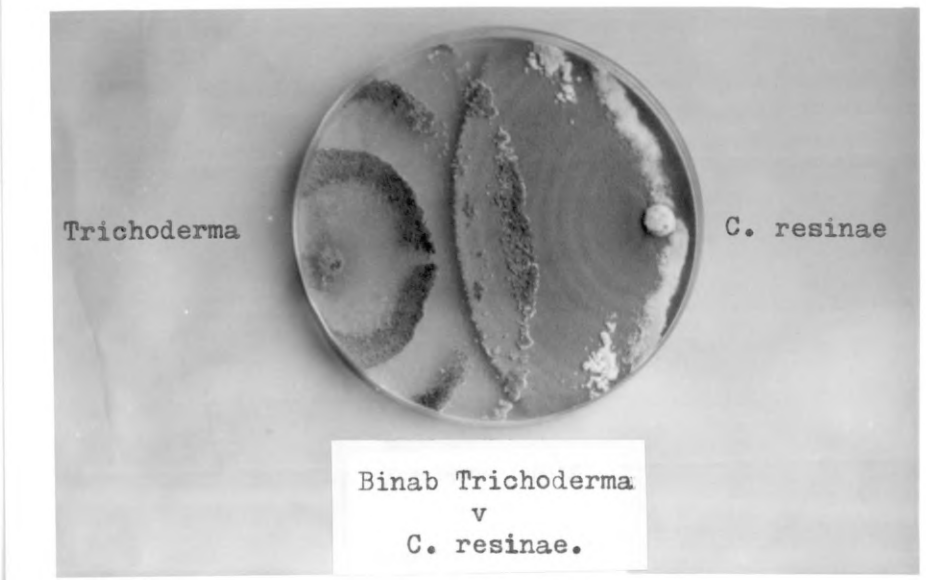


Plate 2.5 Interactive test between Binab *Trichoderma* and *Cladosporium resinae* resulting in an intermingling reaction.

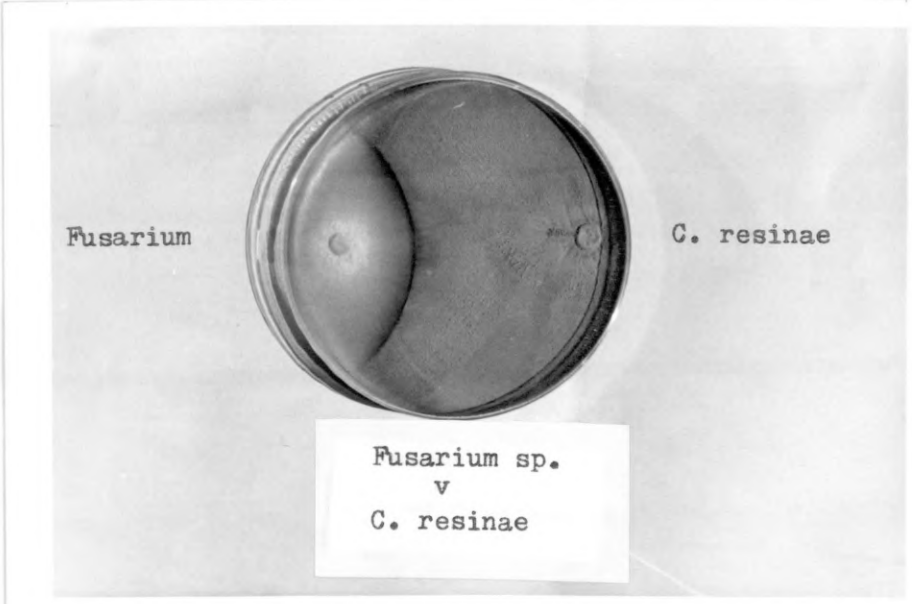


Plate 2.6 Interactive test between *Cladosporium resinae* and a *Fusarium* sp. resulting in a stalemate reaction.

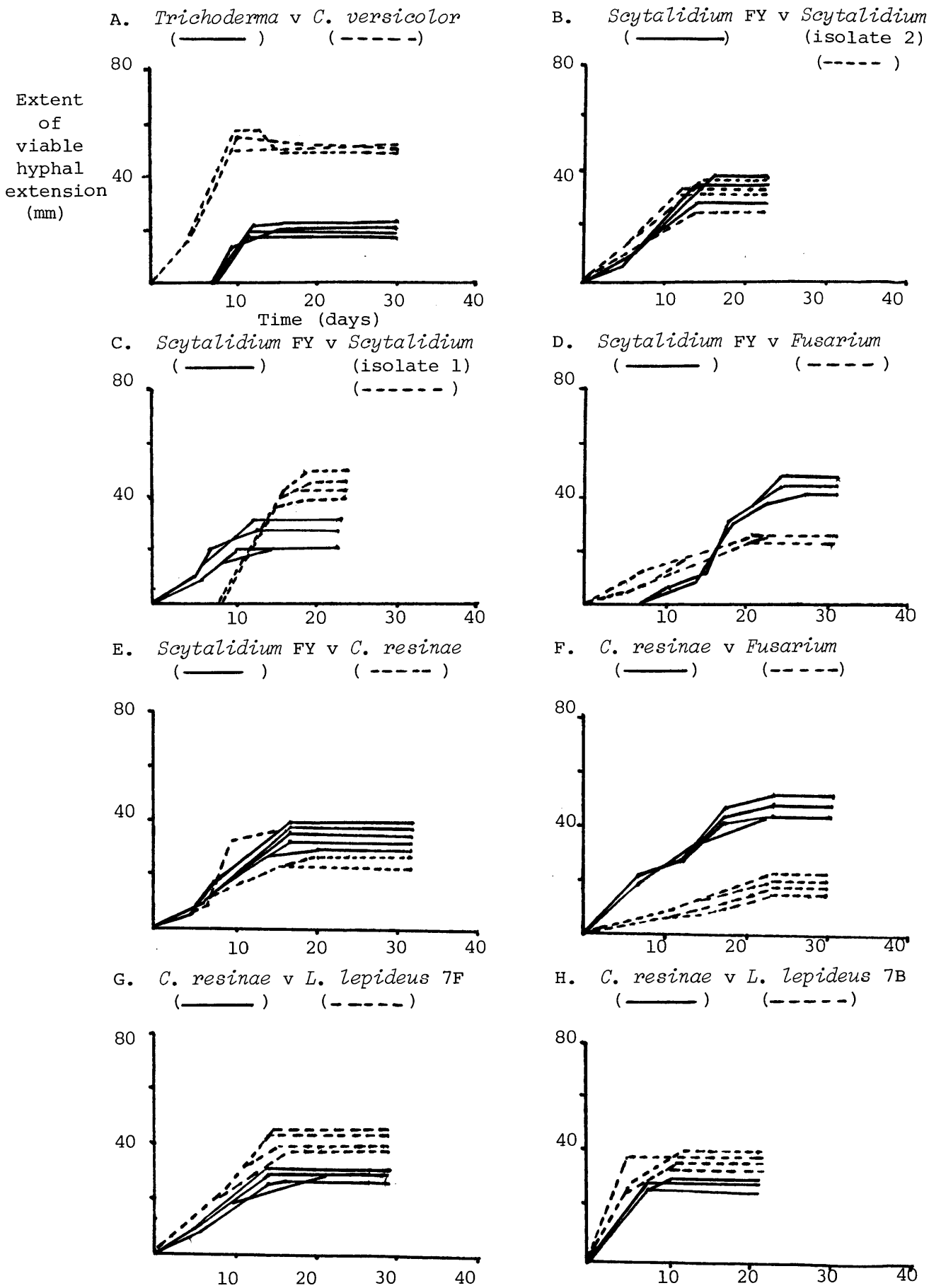


Figure 2.3 Interactions between control fungi, resident pole organisms and basidiomycetes resulting in stalemate reactions, carried out at 25°C.

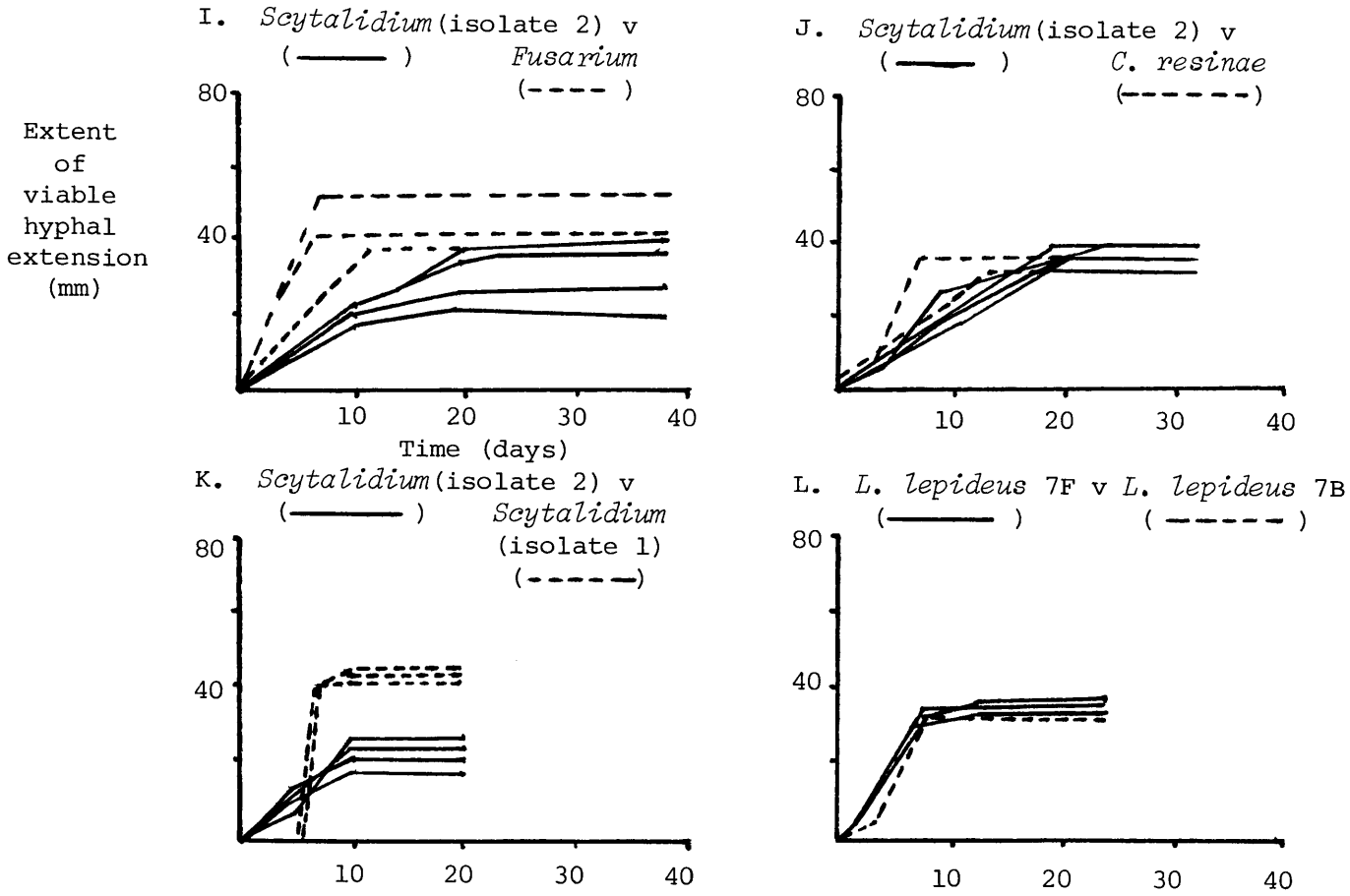


Figure 2.3 Contd.

Target Fungus \ Antagonist Fungus	Binab FYT *Trichoderma*	*Scytalidium* FY strain	*Scytalidium* isolate (1)	*Scytalidium* isolate (2)	*Fusarium* (pole isolate)	*Cladosporium resinae*	*L. lepideus* (FPRL 7F)
L. lepideus (FPRL 7B)	R	R	R	R	R	S	S
L. lepideus (FPRL 7F)	R	R	R	R	R	S	NT
Scytalidium FY strain	I	NT	S	S	S	S	-
Scytalidium isolate (1)	I	S	NT	S	-	-	-
Scytalidium isolate (2)	I	S	S	NT	S	S	-
Fusarium (pole isolate)	I	S	I	S	NT	S	-
Cladosporium resinae	I	S	I	S	S	NT	S
Serpula lacrimans	R						
Fomes annosus	R						
Coriolus versicolor	S						
Coniophora puteana	R						

Table 2.2: The results of interaction tests between target and antagonist fungi (R = replacement and accompanying lysis of test fungi, I = intermingling with accompanying fungistasis of test fungi, S = stalemate between test and antagonist fungi, NT = not tested).

by Rayner and Todd (1979). The three specific reaction types identified during this study (i.e. Replacement, Intermingling and Stalemate) are identical to those found in wood by these other authors and this is especially interesting since the fungi used during this study are common wood colonising or inhabiting organisms. The results presented in this chapter thus contribute further to the body of knowledge on microbial interactions between microfungi and basidiomycetes.

There is always a danger in drawing too close a comparison between wood and agar studies, however the results show that on nutrient rich agar, the biological control organisms present in Binab FYT pellets i.e. *Scytalidium* FY strain and a composite *Trichoderma* culture, are capable of causing the death of both British and German strains of *L. lepideus* at both laboratory temperature (25°C) and at a temperature more closely related to that likely to be found in the field (10°C). Binab FYT pellets were themselves shown to be effective against a strain of *L. lepideus* isolated from creosoted material although this appeared to be entirely due to the *Trichoderma* present in the pellets. Various pole isolate organisms such as *Scytalidium* and *Fusarium* were also found to be capable of killing *L. lepideus*. Interestingly a species of *Penicillium* present as a

contaminant in the laboratory also produced this effect, causing lysis and death of the decay fungus in a manner identical to the biological control organisms. This fact is of interest since it indicates that fungi other than those incorporated in the Binab FYT pellets may be capable of acting as control agents.

The ability of potential control agents to grow and become quickly established in poles, even at the low temperatures likely to be encountered during Autumn and Winter, would be a considerable aid in practice. The results of growth rate studies showed that considerable variation was evident among the organisms used in the study at 5, 10 and 25°C. Of the organisms found to be effective in killing *L. lepideus* during interaction tests, Binab *Trichoderma* and *Scytalidium* (isolate 1) had by far the most vigorous growth rates and would thus appear to be the most promising control agents. *Scytalidium* (isolate 1) was isolated from a decayed pole removed from a distribution line in Scotland and its fast growth rate, especially at low temperature, suggest that it might well be a better replacement for the *Scytalidium* FY strain currently used in the Binab FYT pellets.

The apparent fungistasis produced in *Scytalidium* species during intermingling reactions with Binab *Trichoderma* would seem to suggest that the combination of these two fungi, as in the Binab FYT pellets, is not practical since

the faster growth rate of the *Trichoderma* allied to such fungistatic effects means that the action of the *Scytalidium* FY strain will never be realised. This hypothesis is supported by the fact that only *Trichoderma* has been isolated from FYT pellets incubated on malt extract agar.

Interactions between resident pole fungi and between these organisms and *L. lepideus* almost always resulted in a state of stalemate, and this agrees closely with the compartmentalization of fungi in wood found by Rayner and Todd (1979). One exception to this is the replacement of *L. lepideus* by *Fusarium*. At a purely speculative level, since *Fusarium* spp. were among the most consistently isolated pole inhabitants during this study, they may actually exert some form of natural control against this decay fungus. Such an occurrence could in part account for the apparent variation in longevity amongst similarly located poles even when such poles were adequately creosote treated.

When Binab *Trichoderma* was tested against a range of basidiomycetes it was found to replace all of them except *C. versicolor*, and this has intersting implications for the use of *Trichoderma* as a biological control agent in other problem areas of wood decay. However the environmental and nutrient conditions of wood determine both the type of

fungus responsible for its decay as well as the likelihood of success of any biological control system. Therefore the potential of *Trichoderma* as a biological control agent of basidiomycetes other than *L. lepideus* would require extensive field studies in the particular environment in which any such decay fungus was active.

The mechanism of action of *Scytalidium* isolates has been previously elaborated (Ricard and Bollen, 1968; Klingstrom and Johansson, 1973) and has been shown to be associated with the production of both water soluble and water insoluble antibiotics. The results from this present study agree with this mode of action by *Scytalidium* and soluble antibiotics are implicated by the fact that pigment production and cell wall lysis was observed in *L. lepideus* before contact of the two fungi had occurred. No hyphal interaction was observed between strains of *Scytalidium* and *Lentinus*.

Trichoderma species have a long history of involvement in biological control of plant pathogens during which time many mechanisms have been postulated for their mode of action. These have included, antibiotic production, competition for available nutrients, hyphal interaction and an increased activity of the host's defence system. The results of this present study show that the actions of *Trichoderma* against *L. lepideus* were not due to hyphal

interaction since no penetration or coiling by hyphae was evident nor could competition for nutrients be a problem since *L. lepideus* was established on a nutrient rich medium before being replaced by the *Trichoderma*. Increased activity of hosts defence system is not relevant in this situation and so the action of *Trichoderma* would appear to be due to the action of soluble antibiotics. The fact that lysis and pigment release was seen in *L. lepideus* before contact with the *Trichoderma* may suggest action by water soluble antibiotics, however this may also have occurred as a result of volatiles produced by the *Trichoderma* as may be the state of fungistasis induced in some fungi during intermingling reactions with *Trichoderma*.

During interaction of *L. lepideus* and control organisms the mycelium of the decay fungus was disrupted and lysis took place with accompanying pigment release. An analogous situation was observed during the natural ageing process of cultures of *L. lepideus* on malt extract agar. Reddening of the mycelium with accompanying pigment release is evident and microscopic examination of the hyphae showed total disruption and lysis identical to that found during replacement of this fungus by *Trichoderma*. This apparent coincidence can be clarified by observation of the results of Park (1961) and Park and Robinson (1964; 1967). Park (1961) showed that fungi

produced staling substances which "not only have an inhibitory effect on the fungus but also have positive morphogenetic effects". These substances were shown to cause autolysis and vacuolation of hyphae at high concentrations in older parts of the culture. In a later survey Park and Robinson (1967) classified the staling substance as a fungal hormone controlling the internal water distribution normally associated with cell ageing in fungi. More importantly this staling substance was shown to be active against fungi other than the producer and so may be responsible for fungistatic as well as fungicidal effects against competitor fungi. Park and Robinson (1964) have shown that this staling substance was produced by isolates of *F. oxysporum*, *F. moniliforme*, *P. culmorum*, *F. solani*, *P. expansum* and also by *Trichoderma viride* and that it caused vacuolation in growing tips of *A. niger*. The results of this present study suggest that the *Trichoderma* spp. used may indeed produce such a staling substance since vacuolation and lysis was produced in the hyphae of a range of basidiomycetes during their replacement by *Trichoderma* while in other pole resident organisms only a fungistatic state was induced during intermingling of the two fungi. The fact that identical physiological changes are found in the hyphae of *L. lepideus* during both natural ageing and attack by *Trichoderma* spp. also lends strong support to the

hypothesis that the mechanism of action of *Trichoderma* is via the production of a fungal ageing hormone/ staling substance.

The results presented in this chapter are the results of agar studies and as such are thus not guaranteed to be repeated when these organisms interact in creosoted poles. However both the control organisms i.e. *Trichoderma* and *Scytalidium* FY strain were totally effective in replacing both British and German strains of *L. lepideus*. Although these studies were only a first step in the development of a biological control system to counteract decay by *L. lepideus* the results were considered most encouraging and further studies of these fungi were thus warranted both in wood blocks and in creosoted poles in the field.

CHAPTER 3

PROTECTION OF WOOD BLOCKS BY *TRICHODERMA* AND *SCYTALIDIUM* RESIDUES

3.1 INTRODUCTION

Although remedial treatments for the elimination of *L. lepideus* in creosoted poles in Europe show varing degrees of success (Arsenault, 1973), all involve the use of potentially poisonous or dangerous chemicals which have been of increasing concern to environmentalists. Biological control of decay fungi, using microbial antagonists, has long been suggested as a desirable environmentally acceptable alternative to toxic applications in certain situations provided that such antagonists could be shown to be as efficient as conventional chemical preservatives.

Ricard (1976) proposed the usage of species of *Scytalidium* and *Trichoderma* to control decay in standing creosote treated poles and preliminary studies described in Chapter 2 have shown that both *Scytalidium* and *Trichoderma* are capable of overgrowing and causing lysis of *L. lepideus* when grown in agar culture. Few studies however have been undertaken to monitor antagonism between these organisms and *L. lepideus* in wood.

Oxley (1976) using a wood block system showed that both *Scytalidium lignicola* Pesante and *Trichoderma viride* Pers. ex S. F. Gray can control decay by *L. lepideus* and Morris and Dickinson (1981) have shown that *Scytalidium* FY strain induced death in *L. lepideus* mycelium when grown in competition in sawdust filled tubes. Hulme and Shields (1970) showed that preinoculation of birch blocks with *Trichoderma viride* could inhibit decay by *Poly‿porus adustus*, *P. hirsutus* and *P. versicolor*. These workers evaluated the effects of actively growing antagonists against decay fungi under conditions highly favourable for growth. This would not be the case under field conditions as nutrient starvation might well result in early death of the non-decay producing biocontrol organisms unless supported by considerable nutrient supplement from sources external to the wood. Similarly harsh environmental conditions such as frost, waterlogging, etc. might also produce premature death of control organisms unless resistant strains are used. Nutrient starvation and environmental intolerance would be considerably offset if lethal effects of biological control agents were retained after death and lysis of control organisms, e.g. the residues of dead organisms in themselves conferred protection specific to at least some decay fungi. This effect by *Trichoderma* is well known in Japan with reference to the shiitake fungus *Lentinus edodes* (Berk.) Sing. conventionally

grown on oak bed logs. Furthermore if the protective effects of these residues were retained after leaching, a basis for longer term protection would be provided. Klingstrom and Johansson (1973) showed that wood blocks pretreated with *Scytalidium* species and then subsequently autoclaved could give protection from decay by *Poria vaporaria*, *L. lepideus* and *Coniophora puteana*. This chapter describes laboratory experiments to evaluate residual protective effects of *Trichoderma* and *Scytalidium* against decay of wood by *Lentinus lepideus*.

3.2 MATERIALS AND METHODS

600 ml cuboid glass jars were filled to a depth of 2 cms with 3% malt extract agar (Oxoid No. CM59) to provide a surface of 10 sq cms. These were seeded with either *Trichoderma* spp. isolated from Binab FYT pellets (which contain spores and mycelial fragments of three fungi, *Trichoderma polysporum* (Link ex. Pers.) Rifai, *Trichoderma harzianum* Rifai and *Scytalidium* FY strains) supplied by Stokes Bomford (Chemicals) Ltd., Pershore, Worcester, U.K. or with *Scytalidium* FY strain supplied by Bioinnovation Ab Binab, Sigunta, Sweden. Jars were incubated in the dark at 25°C until the agar surfaces were covered with sporulating mats of mycelium.

Blocks of lime (*Tilia vulgaris* Hayne) and pine (*Pinus sylvestris* L.) measuring 10 mm x 10 mm x 10 mm were oven dried at 103° ± 2°C for 4 hours and weighed before sterilization with ethylene oxide as described by Smith (1965) who showed that this form of sterilization, in contrast with propylene oxide, had no deleterious effects on subsequent decay rates by *Lentinus lepideus*. 15 blocks of lime or pine were randomly placed in each jar in contact with the sporulating mycelium of either *Trichoderma* or *Scytalidium*. Blocks were exposed to *Trichoderma* for 1 month or to *Scytalidium* for 2 months. A combined pretreatment of exposure for 1 month to *Trichoderma* followed by ethylene oxide sterilization and exposure for 2 months to *Scytalidium* was also used.

After exposure to biological pretreatment all blocks were again sterilized using ethylene oxide and half were then subjected to leaching in hot water in a sohlet apparatus for 8 hours. The latter were then dried and resterilized as described previously.

All the blocks pretreated with control fungi were placed on actively growing mycelial mats of *Lentinus lepideus* (Fr.)(FRRL 7B) using a similar glass jar system and then incubated in the dark at 25°C. Controls consisting of blocks exposed to only *Trichoderma*, *Scytalidium* or

Lentinus lepideus were also set up and untreated blocks were extracted with hot water in a soxhlet apparatus for 8 hours to evaluate total soluble nutrient loss during leaching of the wood.

Five replicate blocks were removed from each jar at sampling periods of 4, 8 and 12 weeks, adhering mycelium was removed and blocks were oven dried at 103° ± 2°C to constant weight and reweighed for weight loss determination.

In order to monitor the longevity of any protective effects, pine blocks pretreated with either *Scytalidium* or *Trichoderma* and then exposed to *L. lepideus* for 4, 8 or 12 weeks were (after weight loss determination) resterilized and returned to *L. lepideus* cultures for a further 12 weeks. Blocks which had been initially pretreated with *Scytalidium* were resterilized as before using ethylene oxide however those initially pretreated with *Trichoderma* were autoclave sterilized (due to difficulty in obtaining ethylene oxide) before being returned to *Lentinus lepideus*. After incubation the blocks were dried and reweighed as before in order to calculate weight losses.

3.3 RESULTS

The extent and density of growth of *L. lepideus* mycelium on wood blocks pretreated with control fungi was considerably less than on untreated blocks (Plate 3.1). To ensure that mycelium of the test fungi had completely permeated the wood, several blocks were sectioned and examined microscopically. Although no differentiation could be made between *L. lepideus* and *Trichoderma* mycelium, the *Scytalidium* mycelium was easily identified by the presence of dark arthrospores (Plate 3.2) which were detectable throughout wood blocks.

The results for weight losses at the intermediate 4 and 8 week sampling periods are shown in Tables 3.1 and 3.2. These indicate that little decay is evident up to this time even in control blocks.

Results for weight loss over the entire 12 week experimental period (Table 3.3 and Figure 3.1), show that both *Scytalidium* FY strain and the mixed Binab *Trichoderma* culture can independently protect both lime and pine against decay by *L. lepideus*. This protection is evident even when control organisms are killed and blocks are leached to remove soluble toxic metabolites.

Weight loss values for pine blocks pretreated with either *Trichoderma* or *Scytalidium* and then exposed to *L. lepideus* range from 2.6 - 4.5%. However once these are

Plate 3.1 Extent and Density of growth of *L. lepideus* mycelium on A) Blocks pretreated with *Trichoderma* B) Untreated control blocks.

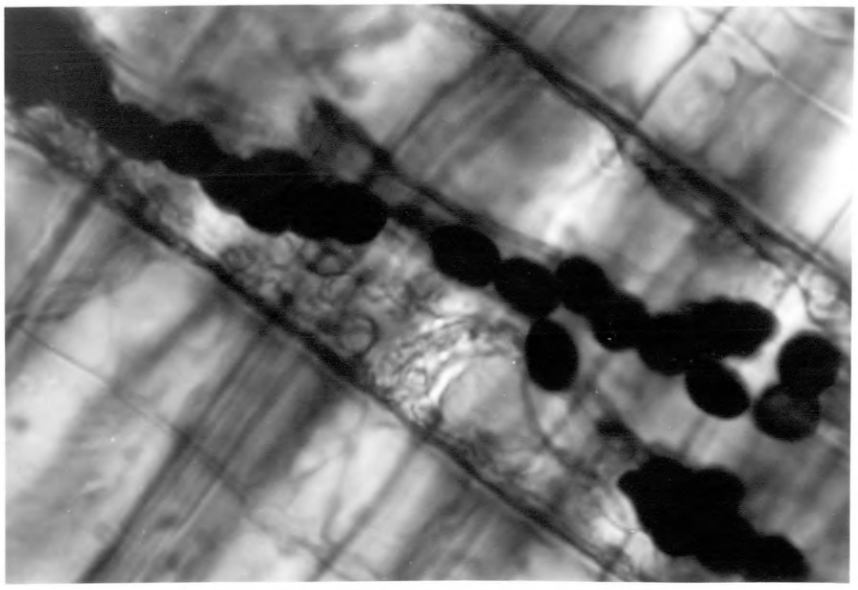


Plate 3.2 *Scytalidium* arthrospores in pine wood (x 1000).

Pretreatments	% Weight Loss	
	Pine	Lime
1. *Trichoderma* 1 month, sterilized	1.7 ± 0.67	2.7 ± 0.29
2. *Trichoderma* 1 month, sterilized, leached	4.0 ± 0.34	4.5 ± 0.35
3. *Scytalidium* 2 months, sterilized	1.5 ± 0.54	2.2 ± 0.48
4. *Scytalidium* 2 months, sterilized, leached	3.7 ± 0.71	1.6 ± 0.45
5. *Trichoderma* 1 month, sterilized *Scytalidium* 2 months, sterilized	2.1 ± 0.16	2.4 ± 0.36
6. *Trichoderma* 1 month, sterilized *Scytalidium* 2 months, sterilized, leached	4.4 ± 0.16	5.9 ± 0.80
Controls (untreated with *Lentinus*)		
7. *Trichoderma* 2 months	3.0 ± 0.39	5.1 ± 0.22
8. *Scytalidium* 3 months	3.3 ± 0.91	3.4 ± 0.39
9. *Trichoderma* 1 month, sterilized *Scytalidium* 3 months	5.4 ± 0.14	5.4 ± 0.28
10. *L. lepideus* 1 month	3.0 ± 0.18	1.9 ± 0.34

Table 3.1 Mean % weight losses and standard errors produced after 4 weeks by *Lentinus lepideus* in pine and lime blocks pretreated with combinations of *Trichoderma* and *Scytalidium*.

Pretreatments	% Weight Loss	
	Pine	Lime
1. *Trichoderma* 1 month, sterilized	1.0 ± 0.30	4.5 ± 0.51
2. *Trichoderma* 1 month, sterilized, leached.	2.1 ± 0.38	2.9 ± 3.7
3. *Scytalidium* 2 months, sterilized	2.6 ± 0.34	2.5 ± 0.32
4. *Scytalidium* 2 months, sterilized, leached	3.6 ± 0.46	2.9 ± 0.55
5. *Trichoderma* 1 month, sterilized *Scytalidium* 2 months, sterilized	4.7 ± 0.88	5.9 ± 0.44
6. *Trichoderma* 1 month, sterilized *Scytalidium* 2 months, sterilized, leached.	5.0 ± 0.35	5.3 ± 0.33
Controls (untreated with *Lentinus*)		
7. *Trichoderma* 3 months	2.1 ± 0.13	4.2 ± 0.31
8. *Scytalidium* 4 months	4.2 ± 0.47	3.9 ± 0.60
9. *Trichoderma* 1 month, sterilized *Scytalidium* 4 months	6.6 ± 0.33	7.3 ± 0.69
10. *L. lepideus* 2 months	4.7 ± 0.42	2.4 ± 1.18

Table 3.2 Mean % weight losses and standard errors produced after 8 weeks by *Lentinus lepideus* in pine and lime blocks pretreated with combinations of *Trichoderma* and *Scytalidium*.

Pretreatments	% Weight Loss	
	Pine	Lime
1. *Trichoderma* 1 month, sterilized	2.6 ± 0.46**	5.9 ± 0.21†
2. *Trichoderma* 1 month, sterilized, leached	3.6 ± 0.67*	4.5 ± 0.46*
3. *Scytalidium* 2 months, sterilized	3.1 ± 0.34**	4.9 ± 0.16*
4. *Scytalidium* 2 months, sterilized, leached	4.5 ± 0.52*	4.5 ± 0.32*
5. *Trichoderma* 1 month, sterilized *Scytalidium* 2 months, sterilized	5.8 ± 0.73*	6.6 ± 0.61†
6. *Trichoderma* 1 month, sterilized *Scytalidium* 2 months, sterilized, leached	6.1 ± 0.26*	7.7 ± 0.52†
Controls (untreated with *Lentinus*)		
7. *Trichoderma* 4 months	4.5 ± 0.51*	5.2 ± 0.15†
8. *Scytalidium* 5 months	5.2 ± 0.47*	4.0 ± 0.36*
9. *Trichoderma* 1 month, sterilized *Scytalidium* 5 months	6.0 ± 0.21*	7.2 ± 0.42†
10. *L. lepideus* 3 months	12.7 ± 3.03	23.8 ± 8.08
11. Soxhlet leaching	1.5 ± 0.38	2.2 ± 0.07

Table 3.3 Mean % weight losses and standard errors produced after 12 weeks by *Lentinus lepideus* in pine and lime blocks pretreated with combinations of *Trichoderma* and *Scytalidium*. 'T' values indicate significances of differences between weight losses of blocks pretreated with control organisms and those of controls treated with *L. lepideus* only. (**, significaint at 1%; *, significant at 5%; †, significant at 10%).

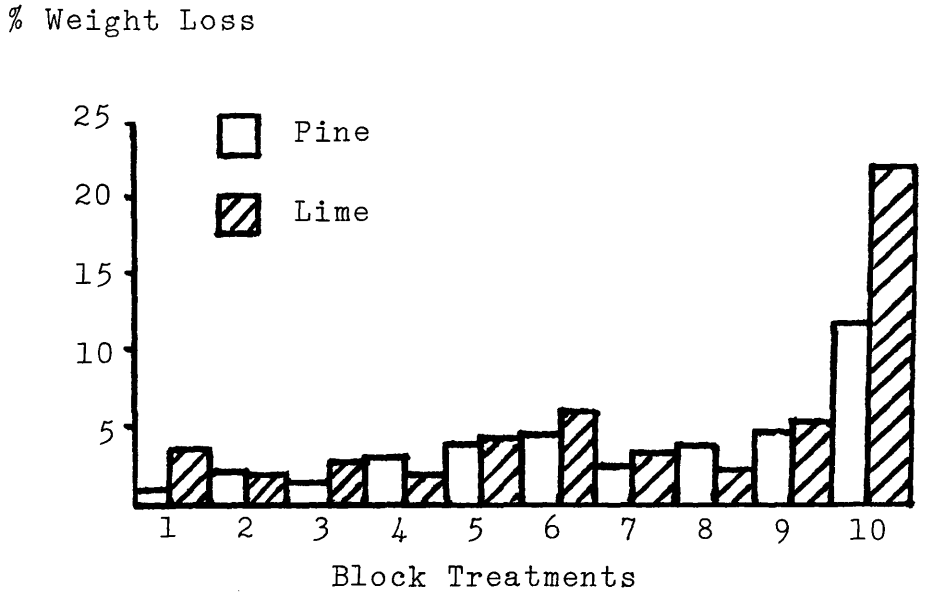


Figure 3.1 % weight losses in pine and lime blocks after exposure to *L. lepideus* for 12 weeks. Values are corrected for losses of soluble materials during leaching and treatments are numbered as in Table 3.1.

corrected for the extractable fraction in pine (1.5%) they are reduced to less than 3.0%. During the same twelve week period untreated pine blocks subjected to *L. lepideus* attack lost an average 12.7% of their weight. Wide interblock variation in weight losses (5 - 24%) was observed in blocks exposed only to *L. lepideus* but was not evident in blocks subjected to biological pretreatment, maximum variations of which ranged from 3.5 - 6.0%. Analysis of variance of mean weight loss values of pretreatment blocks and controls show that these are very significantly different ($F = 9.00$, $p = 0.001$).

Weight loss values for lime blocks pretreated with either *Scytalidium* or *Trichoderma* and then exposed to *L. lepideus* were less than 3.7% when corrected for the extractable fraction for lime (2.2%). During the same period untreated blocks subjected to *L. lepideus* attack lost 23.8% of their weight. Wide interblock variation in weight losses (3-40%) were again observed in blocks exposed only to *L. lepideus* but were not found between blocks in pretreatment groups (3.3 - 5.7%). Analysis of variance again showed that the mean figures for pretreatment groups and those of controls are very significantly different ($F = 5.26$, $p = 0.001$). 'T' tests were carried out on corrected weight loss figures to evaluate the

degrees of significance of differences between individual pretreatment groups and controls in both lime and pine and these are indicated on Table 3.3.

Combined treatments of *Trichoderma* and *Scytalidium* always resulted in larger weight losses than blocks treated with either of the two separately although these never exceeded corrected values of 4.6% in pine and 5.5% in lime and were probably the result of the longer incubation periods (up to 6 months) to which these blocks were exposed.

Pine blocks originally pretreated with *Scytalidium* before being exposed to *L. lepideus* for 4, 8 and 12 weeks, resterilized and re-exposed to the decay fungus for a further 12 weeks resulted in average weight loss values ranging from 4-6%. These figures show only a marginal increase from the 4.5% recorded after the original 12 week exposure to *Lentinus*. However, blocks pretreated with *Trichoderma* and exposed to *L. lepideus* before being autoclave sterilized and re-exposed to the decay fungus resulted in an apparent loss of protection, average weight loss values ranging from 5.2 - 59.8% (Table 3.5).

Pretreatments	% Weight loss in Pine Blocks
1. *Trichoderma* 1 month, sterilized, *Lentinus* 1 month, autoclaved.	59.8 ± 1.27
2. *Trichoderma* 1 month, sterilized, *Lentinus* 2 months, autoclaved.	43.8 ± 4.58
3. *Trichoderma* 1 month, sterilized, *Lentinus* 3 months, autoclaved.	49.9 ± 5.33
4. *Trichoderma* 1 month, sterilized, leached, *Lentinus* 1 month, autoclaved.	12.6 ± 4.13
5. *Trichoderma* 1 month, sterilized, leached, *Lentinus* 2 months, autoclaved.	5.2 ± 0.24
6. *Trichoderma* 1 month, sterilized, leached, *Lentinus* 3 months, autoclaved.	9.8 ± 4.16
7. *Scytalidium* 2 months, sterilized, *Lentinus* 1 month, sterilized.	4.3 ± 0.57
8. *Scytalidium* 2 months, sterilized, *Lentinus* 2 months, sterilized.	4.1 ± 0.43
9. *Scytalidium* 2 months, sterilized, *Lentinus* 3 months, sterilized.	4.0 ± 0.22
10. *Scytalidium* 2 months, sterilized, leached, *Lentinus* 1 month, sterilized.	6.0 ± 0.72
11. *Scytalidium* 2 months, sterilized, leached, *Lentinus* 2 months, sterilized.	5.4 ± 0.52
12. *Scytalidium* 2 months, sterilized, leached, *Lentinus* 3 months, sterilized.	5.3 ± 0.51

Table 3.5 Mean % weight losses and standard errors produced after 12 weeks by *Lentinus lepideus* in pine blocks pretreated with combinations of *Trichoderma*, *Scytalidium* and *Lentinus*.

3.4 DISCUSSION

The experiments described in this Chapter were undertaken using small blocks and short incubation periods to facilitate rapid processing of large numbers of replicates and pretreatments. An incubation temperature of 25°C was used to optimise growth of *L. lepideus* the strain of which used was different to that specified in European standard EN 113. Because of this different methodology, results are not directly comparable with those produced using 113 test procedures. The decay values for untreated lime blocks are very acceptable for a twelve week incubation period and clearly show that biological control of the decay process occurred. Control of decay in pine was also clearly demonstrated although the strain of *L. lepideus* used was either less virulent in pine than in lime or alternatively lime was more decay susceptible under the test conditions.

Previous work carried out using weight loss as an indicator of control of *L. lepideus* by immunizing commensal organisms (Oxley, 1976) examined interaction between living fungi, thus no restrictions were placed on modes of action of control agents. Oxley's results showed that both *S. lignicola* and *T. viride* could control *L. lepideus* although he found *S. lignicola* to be more effective.

The results presented here confirm Oxley's results for *Trichoderma* and show that both *Trichoderma* and *Scytalidium* FY strain exert control over *L. lepideus*. However the results presented in this thesis show for the first time that control by residues of both organisms persists even when the control agents had been killed and the wood blocks thoroughly leached. Furthermore both of the control organisms were found to be equally effective.

While the active protective mechanism of *Scytalidium* in wood is well established and has been attributed to the production of both water soluble, water insoluble and heat stable antibiotics, less attention has been placed on the mode of action of *Trichoderma* in wood protection. *Trichoderma* species have long been suggested for usage as biocontrol agents of plant pathogens (Allen and Haenseler, 1935; Aytoun, 1952; Ricard, 1970; Sierota, 1977; Lin and Cook, 1979; Smith *et al*, 1981) and have been suggested for usage as control agents for post harvest deterioration of freshly felled wood by basidiomycetes (Hulme and Shields, 1970). Lundborg and Unestam (1980) have successfully used *Trichoderma harzianum* for the control of *Fomes annosus* (Fr.) Cooke in Norway spruce.

Although Dennis and Webster (1971 a and b) showed that very large interstrain and interspecies differences existed

between members of the genus *Trichoderma* with regard to their ability to produce volatile and non-volatile antibiotics against a wide range of fungi, no mechanism for the action of *Trichoderma* sp. against *L. lepideus* has been shown to date. As these results show residual protection after death and thorough leaching of *Trichoderma* treated blocks, it may be assumed that the mode of action cannot be by competition for available nutrients nor by hyphal interaction of viable mycelia, reinforcing the results found during agar interaction studies. Since blocks, pretreated with *Trichoderma* and subsequently thoroughly leached, still retained active protection from decay by *L. lepideus* it would appear that the mechanism of action of *Trichoderma* was not by the production of water soluble antibiotics conventionally assumed to be associated with *Trichoderma* inhibition unless these were bound within the wood. The mechanism of action of *Trichoderma* does however seem to be heat labile since the protective mechanism was lost in *Trichoderma* treated blocks after autoclaving. This might imply that some protective metabolite is itself heat labile or that changes in the wood structure brought about by autoclaving result in release of the protective element from the blocks or make the wood inately more decay susceptible. It has already been shown (Klingstom and Johansson, 1973) that protective effects of *Scytalidium* spp. are due to the production of heat stable antibiotics.

The results presented in this chapter show for the first time that protection of wood from decay by *L. lepideus* can be produced by either *Scytalidium* FY strain or *Trichoderma* residues even after death and removal of soluble remains of these organisms. While further work with a number of strains of *Lentinus* is required, the effects reported in this chapter in combination with competitive growth, hyphal interaction and antibiotic production by viable organisms within creosoted poles would suggest that biological control may have considerable potential for development as an appropriate prophylactic treatment against *L. lepideus*.

CHAPTER 4

MECHANISM OF ACTION OF *TRICHODERMA*

4.1 INTRODUCTION

Although *Trichoderma* is probably the most widely used fungus in biological control of plant pathogens, precise mechanisms involved in prevention of wood decay have not received as much attention as have those of *Scytalidium*. Consequently little work has been undertaken on modes of action of *Trichoderma* against *L. lepideus* although it is interesting to note that growth of the edible shiitake mushroom *Lentinus edodes* (Berk) Sing. is completely inhibited in oak logs if these have been pre-infected with *Trichoderma* (Komatsu, 1976).

Suggested prophylactic mechanisms of *Trichoderma* involved in plant pathogen control have included: production of antibiotics (Allen and Haenseler, 1935; Godtfredson and Vangedal, 1965; Komatsu and Inada, 1969; Dennis and Webster, 1971a; Sierota, 1977; Ishakawa, Nagao, Oki and Kawabe, 1980), direct competition for available nutrients (Chang and Kommedahl), 1968; Hulme and Shields, 1970; Lin and Cook, 1979) hyphal interaction (Aytoun, 1952; Ricard, 1970; Dennis and Webster, 1971c; Elad *et al*, 1983), and suppression of pioneer colonisers which by their actions had reduced the plant's defence mechanisms (Smith, Blanchard and

Shortle , 1981); which mechanisms may act separately or simultaneously.

However it is obvious from the results presented in Chapter 3 which showed that the presence of residues of killed *Trichoderma* in wood also inhibited *L. lepideus* and also observations made during agar studies (Chapter 2) that such mechanisms as competition for nutrients, hyphal interaction and increased host defences are not necessarily involved in any wood protection conferred by this fungus.

The genus *Trichoderma* was first introduced into mycological literature by Persoon in 1794 to accommodate four fungal species now commonly considered to be unrelated to one another (Rifai, 1969). From that date until the publication of 'A revision of the genus *Trichoderma*' by Rifai in 1969 much controversy has taken place regarding the identity of this and closely related genera. The history of the genus *Trichoderma* as described by Rifai (1969) and extracted below illustrates the problems which have arisen during the classification of species of this fungus. From the first description of *Trichoderma* by Persoon until the 1830's many species had been encountered and assigned to this genus. Fries (1829) considered that all fungi which produced green spores were strains of *Trichoderma viride* Pers. ex Fr. and he reduced to synonymy other organisms such as the genus *Aleurisma*. Furthermore it was commonly believed that *Trichoderma* belonged to the

Gasteromycetes however the Tulasne brothers (1865) made the important observation that *Trichoderma* was in fact the conidial stage of *Hypocrea rufa* (Pers. ex Fr.) Fr. This fact has serious implications on the further classification of *Trichoderma*.

Harz (1871) was the first mycologist to emphasise the importance of microscopic characters as a yardstick for delimiting the genus and he agreed with Fries and previous workers that the genus had only one member. Vuillemin (1887) transferred *Trichoderma viride* to the genus *Acrostalagmus* Corda which in recent years is considered to be synonymous with *Verticillium*, a fungus similar to *Trichoderma* in its structural characteristics. The idea that *Trichoderma* was a monotypic genus remained until early in the 20th century when Oudemans and Koning (1902), Waksman (1916) and Abbott (1926) isolated and identified as different up to four species of *Trichoderma*.

This situation prompted Birsby (1939) to investigate the genus *Trichoderma* and while he accepted the genetic connection with *Hypocrea rufa* he stated that he could not distinguish between mono-ascospore cultures of *H. rufa* and those of *H. gelatinosa*. Birsby concluded that *Trichoderma* was a monotypic genus and that *H. rufa* and *H. gelatinosa* were in effect different forms of the same species.

Webster (1964) showed that the *Trichoderma* states of various Hypocrea species could be distinguished from one another by microscopic examination of differences in their morphological characteristics and this led Rifai (1969) to state that *Trichoderma* is not in fact a monotypic genus but one composed of a number of closely related species. Due to the slight morphological differences between species Rifai (1969) concluded that the numbers of species identified by him should not be regarded as a complete toxonomic treatment of the genus *Trichoderma*. Because of the close similarities between strains of *Trichoderma* Danielson and Davey (1973a and b) have suggested more recently that it might be more practical to classify *Trichoderma* species by either their temperature growth ranges or by their carbon and nitrogen growth requirements.

One outcome of the confused classification of the genus *Trichoderma* is that many cases of its use as a fungal antagonist and in particular its ability to produce antibiotics cited in the literature cannot be accepted with total confidence. As Birsby (1939) had stated that *Trichoderma* was a monotypic genus, Brian (1944), Brian and Hemming (1945), Brian and McGowan (1945) and Brian, Curtis and Hemming (1946) described the production of gliotoxin and viridin by strains of what these authors called *T. viride*. However Webster and Lomas (1964) later showed that the organism used was actually

Gliocladium virens Miller, Giddens and Foster a fungus which closely resembles *Trichoderma* in its phialide disposition. Another case of mistaken fungal identity was that of Weindling (1932), who observed the production of a "mould toxin" by *Trichoderma lignorum*. However Weindling (1941) admitted that the toxin producing fungus was *Gliocladium* and not the *Trichoderma* spp. first suspected.

The ability of true *Trichoderma* strains to produce antibiotic substances has been well documented (Dennis and Webster, 1971a; Godtfredson and Vangedal, 1965; Park and Robinson, 1964). Although the results of these and other experiments might support the observations of many of the earlier mycologists that *Trichoderma viride* produced antibiotics some of their recorded findings were almost certainly due to unrelated organisms.

Mowe, King and Senn (1983) demonstrated that fungi respond tropically by positive or negative directional growth to volatiles emanating from wood baits. It was also shown that *Chaetomium globosum*, normally stimulated by volatiles to grow towards unpreserved pine, may grow away from such baits if these are treated with some preservatives. All these responses may be significantly modified if contaminant microorganisms, including *Trichoderma* spp. are located at baits (King,

Mowe, Smith and Bruce, 1981). These results support observations by Dennis and Webster (1971b) that strains of *Trichoderma* produce volatile compounds which inhibit growth of a range of fungi imperfecti and also the basidiomycete *Fomes annosus* and that radial fungal growth may be inhibited or stimulated by microbial volatiles (Dick and Hutchinson, 1966).

The fact that volatile induced reactions occur between fungi and wood and between fungi and other microorganisms adds a causal dimension to the mechanics of microbial succession. An ability to locate new substances and demonstrate accelerated growth towards them as a response to volatile stimulation should be a considerable competitive aid in microbial succession as should the production of volatiles active against other competitors. The capacity of some fungi to detect sub-lethal volatile levels and grow towards less toxic concentrations by orientated growth is also obviously a useful survival aid. In any case, volatile determined interactions between fungi have interesting implications for biological control of decay fungi in wood especially if volatile metabolites are species specific.

The aims of the experiments described in this chapter are:-

1) To determine if *Trichoderma* and *Scytalidium* strains commercially included in Binab FYT pellets could produce non-volatile antibiotic compounds active against the decay basidiomycete *Lentinus lepideus*.

2) To determine if such *Trichoderma* strains produced volatiles active against *L. lepideus*.

3) To detect any tropic responses by *L. lepideus* to volatile metabolites.

4) To identify the chemical components responsible for any volatile activity produced.

5) To examine if mould fungi, commonly found in creosoted transmission poles, inhibited the growth of *L. lepideus* by volatile production.

6) To determine if microbially produced volatiles inhibited a broad range or a restricted group of wood decay basidiomycetes.

7) To determine if protection of wood blocks by *Trichoderma* residues was due to volatiles emanating from such residues.

4.2 MATERIALS AND METHODS

4.2.1 Non-volatile antibiotic production

Conical flasks (500 mls), containing 100 mls of 3% malt extract broth were inoculated with two 8 mm discs cut

from the margin of vigorously growing cultures of *Trichoderma polysporum*, *T. harzianum*, *Scytalidium* FY strain and a *Trichoderma* sp. isolated from Binab FYT pellets. The flasks were incubated in the dark in an orbital incubator (150 r.p.m.) at 25°C. After periods of 7 days for the *Trichoderma* strains and 14 days for the *Scytalidium* the culture liquids were filtered through Whatman No.1 filter paper to remove large hyphal fragments before being filtered under pressure through a micropore filter (pore size - 0.2µm) using a Sartorius membrane filtration unit. 10 mls of this culture filtrate was added to an equal volume of strengthened agar (3% Malt Extract Agar + 0.9% Agar-Agar) which was then poured into a petri dish to give a solid medium which would then contain any antibiotic substances produced. Three replicates were prepared for each test.

Control plates were prepared using uninoculated broth incubated under the same conditions as inoculated flasks. 8 mm discs, cut from the margins of actively growing cultures of *Lentinus lepideus* FPRL 7B, and *L. lepideus* FPRL 7F were then centrally inoculated onto both test and control plates. The plates were then incubated in the dark at 25°C for 8 days.

As both *Trichoderma* and *Scytalidium* FY strain are incorporated in the Binab FYT pellets, to test the

compatability of the two fungi, *Scytalidium* FY strain was inoculated onto plates produced from *Trichoderma* filtrates. The results were recorded as the differences in mean radial growth of fungi on test plates and controls as a % of the latter at a time when fungi reached the periphery of control plates, usually 8 days for the *Lentinus* strains and 10 days for *Scytalidium*.

Since the results of Chapter 2 showed that the residual protective effect of *Trichoderma* was lost after autoclaving of pretreated wood blocks a set of 3 test and control plates were set up as above. However in this case the *T. harzianum* filtrate used was autoclaved for 15 mins at 105°C prior to being added to the strengthened agar. The plates were then inoculated as before with *L. lepideus* FPRL 7F and incubated at 25°C for 8 days.

To ensure that any inhibitory effects on the growth of *L. lepideus* were due to soluble antibiotic production by *Trichoderma* and not volatiles which had become submerged and trapped in the culture filtrate N_2 gas was bubbled through a sterile culture filtrate of FYT pellet *Trichoderma* for up to 6 hours in order to remove any volatiles physically trapped in the sample. Plates were then prepared and inoculated as before with *L. lepideus* FPRL 7F and incubated at 25°C for 8 days.

4.2.2 Volatile antibiotic production

The technique of Dick and Hutchinson (1966) was used to evaluate volatile production by the following test organisms: *Penicillum chrysogenum* Thom , *Aspergillus niger* Van Tieghem, *Cladosporium resinae* (Lindau) de Vries (isolated from creosoted wood at this laboratory) and a *Fusarium* sp. isolated in similar fashion. Cultures of *Trichoderma polysporum* (Link ex Pers) Rifai (CMI 206039), *Trichoderma harzianum* Rifai (CMI 206040), a *Trichoderma* culture isolated from Binab FYT pellets (which contain spores and mycelial fragments of three fungi, *Trichoderma harzianum*, *Trichoderma polysporum* and *Scytalidium* FY strain) supplied by Stokes Bomford (Chemicals) Ltd., Pershore, Worcester, U.K., and *Scytalidium* FY strain supplied by Bioinnovation Ab-Binab, Sigtuna, Sweden, were also included in the study.

Agar cores (8 mm diam.) were aseptically removed from the actively growing margins of test fungi and were placed in the centres of petri dishes containing 15 mls of 3% malt extract agar. Plates inoculated with cores removed from actively growing cultures of *Lentinus lepideus* (Fr.) (FPRL 7B) and *L. lepideus* (FPRL 7F) in a similar manner were then inverted over plates inoculated with potential antagonists. A thin polythene membrane was placed between the two dishes which were then sealed together with adhesive tape (Figure 4.1).

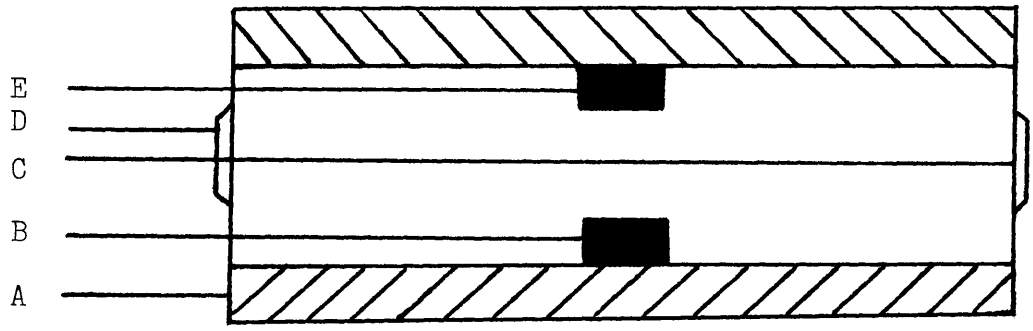


Figure 4.1 Experimental system used to test volatile production by candidate biological control fungi (Dick and Hutchinson, 1966).

A - Growth medium in petri dish (3% malt extract agar)

B - Inoculum of potential antagonist

C - Polythene membrane

D - Sellotape seal

E - Inoculum of target organism in inverted petri dish

Control plates consisted of *L. lepideus* plates inverted over uninoculated malt agar dishes. Four replicate units were set up for each interaction study and these were incubated in the dark at 25°C. Units were also set up in which candidate antagonists were placed above colonies of *L. lepideus* to determine if positioning of plates had any influence on interactions. Test units were examined daily and the incubation periods required for production of mean colony radii of 35 mm (colony diameters of 70 mm) by the *L. lepideus* colonies were noted.

Test units using the *Trichoderma* culture isolated from Binab FYT pellets, *Trichoderma polysporum* and *Trichoderma harzianum* were also set up with the basidiomycetes *L. lepideus* (FPRL 7B), *L. lepideus* (FPRL 7F), *Serpula lacrimans* (Wulf ex Fr.) Schroeter (FPRL 12C), *Coniophora puteana* (Sch. ex Fr.) Koreten (FPRL 11E), *Fomes annosus* (Fr.) Cooke (FPRL 41E), and *Coriolus versicolor* (Lin. ex Fr.) Quélet (FPRL 28A) and growth was monitored in similar fashion at both 10 and 25°C. Growth rates of the fungi were too slow to enable the tests to be carried out at 5°C.

Test units in which the *Trichoderma* culture isolated from FYT pellets, *T. polysporum* and *T. harzianum* were monitored for volatile production active against

Scytalidium FY strain were also set up to test the compatability of the two types of fungi to act together as biocontrol agents as incorporated in the Binab FYT pellet. To quantify the degree of inhibition produced in volatile susceptible basidiomycetes and the influence of volatile concentration gradients on fungal growth, experiments involving *L. lepideus* FPRL 7F, *L. lepideus* 7B, and *F. annosus* interactions with *T. harzianum*, *T. polysporum* and the *Trichoderma* culture isolated from Binab FYT pellets were also set up. 8 mm cores were removed from the margins of actively growing cultures (2 weeks) of *L. lepideus* and *F. annosus* and placed in the centres of plates of 3% malt extract agar. Benomyl was incorporated at concentrations of 4 ppm and 1 ppm in media used to support *L. lepideus* and *F. annosus* respectively. The addition of benomyl at these concentrations permitted growth by basidiomycetes but not *Trichoderma* spp. (Clubbe and Levy, 1977). Three 8 mm cores of the candidate control fungi were placed on pre-sterilized, plastic supports equidistant (30 mm) from basidiomycete inocula (Plate 4.2). The supports prevented contact between media used for growth of candidate control fungi and test fungi. In some cases antagonists were located at distances of 10 or 20 mm from basidiomycete inocula. In others only one antagonist inoculum was placed with basidiomycetes in order to determine if tropic responses to volatile emanations from biocontrol fungi were produced. Five replicate plates were set up for each

interactive study and controls consisted of plates inoculated with basidiomycetes in which antagonistic fungi were replaced by cores of uninoculated agar. All plates were incubated in the dark at 25°C and were examined daily for growth of fungi. Results were recorded as differences in mean radial growth of basidiomycetes towards antagonists and controls as a % of the latter at the time when basidiomycetes reached control locations, usually, 8 days for *L. lepideus* FPRL 7F and *Fomes annosus* and 9 days for *L. lepideus* FPRL 7B.

Using the above methodology a supplementary experiment was set up to monitor possible volatile emanation from *Trichoderma* residues, the agar cores containing candidate control fungi being replaced by wood blocks (10 mm x 10 mm x 10 mm) pretreated with *Trichoderma* isolated from Binab FYT pellets. Test blocks contained either live *Trichoderma*, killed *Trichoderma* or the remains of killed *Trichoderma* after aqueous leaching, and controls consisted of untreated wood blocks. Three blocks were placed on plastic supports equidistant (30 mm) from a control *L. lepideus* (FPRL 7B) inoculum. As above 5 replicate plates were set up for each test and all plates were again incubated in the dark at 25°C.

4.2.3 Identification of volatile components

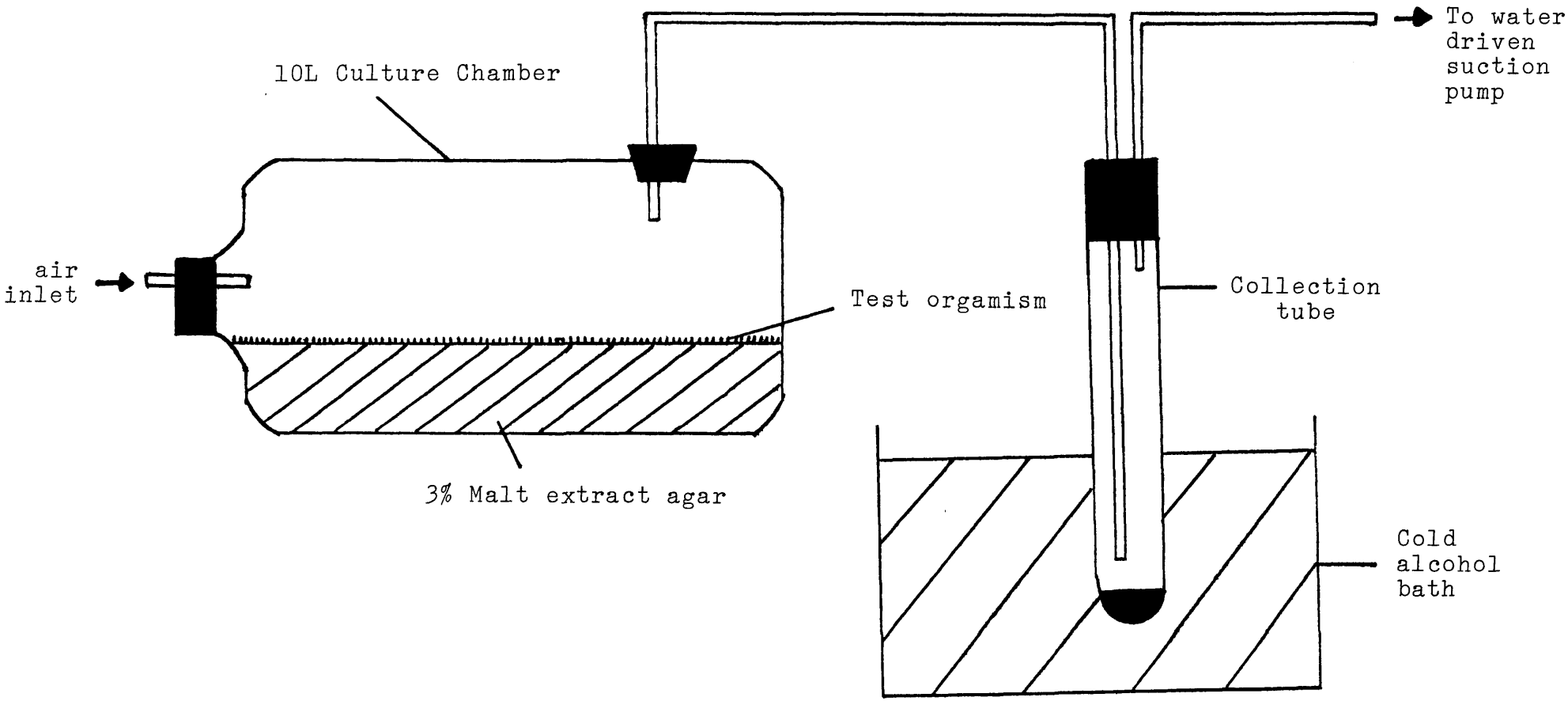
As the results from experiments carried out in the previous section showed that only the *Trichoderma* species

tested produced volatiles active against *L. lepideus* a supplementary experiment was set up to tentatively identify the chemical components responsible. Binab *Trichoderma* and *A. niger* were selected for study since they respectively represented producers and nonproducers of volatiles active against *L. lepideus*.

To investigate the chemical nature of the volatiles a method of collecting these based on that used by Dennis and Webster (1971b) was devised. For each organism a bell shaped glass jar (volume, 10L) containing 4.5L of 3% malt extract agar was prepared. After autoclaving at 120 °C for 15 minutes the jars were placed on their sides before the agar media had set. Each jar was inoculated with cores of agar removed from the growing margins of cultures of either Binab *Trichoderma* or *A. niger*. The jars were incubated in the dark at 25°C and *Trichoderma* and *A. niger* were grown for 7 and 10 days respectively before the vapours were collected by passing them through a trap immersed in a bath of refrigerated alcohol (-15 °C) using a water driven suction pump. The collection was carried out for 8 hours after which the trap was sealed and the volatiles, now frozen in a solid state, were stored in a deep freeze at -15°C. (Figure 4.2)

Collections of volatile compounds were analysed using a Hewlet/Packard 5710A Gas Chromatograph and Hewlet/ Packard 3390A integrator. Collections of volatiles were

Figure 4.2 Collection of volatile metabolites from agar cultures of Binab *Trichoderma* and *A. niger*.



removed from the deep freeze and allowed to thaw slowly after which vapour samples were removed by syringe and injected into the chromatograph. As suggested by Robinson and Garrett (1969) retention times were used to establish the identity of compounds using two columns packed with two different substrates having dissimilar polarity characteristics. One column, 1.54 m long, was packed with 15% (w/w) Carbowax 1500 on celite and operated at 140°C with an injection temperature of 150°C. The second shorter column was packed with silicone oil on celite and was operated at 50°C with an injection temperature of 150°C. The following standards were tested to establish whether they were present in the volatile fractions collected from the fungi; Acetaldehyde, n-butyraldehyde, propionaldehyde, acetone, ethyl acetate and isobutyl alcohol. These chemicals have been shown (Robinson and Garrett, 1969) to be present in the vapours of fungi.

4.3 RESULTS

4.3.1 Non-volatile antibiotic production

Table 4.1 shows percentage inhibition of hyphal extension of *L. lepideus* 7B, *L. lepideus* 7F and *Scytalidium* FY strain when grown on plates seeded with antibiotic substances from either *Trichoderma* spp. or *Scytalidium* FY strain. As radial hyphal measurements were made in two directions on each test plate and three replicate plates were used, each value is a mean of six readings. Colony area was calculated from radial growth

Target Fungus / Antibiotic Producer	*L. lepideus* (FPRL 7B)	*L. lepideus* (FPRL 7F)	*Scytalidium* FY strain
Binab FYT *Trichoderma*	87.8 (96.3)	54.8 (74.9)	55.1 (74.1)
Trichoderma polysporum	33.0 (50.5)	38.7 (58.1)	61.4 (79.7)
Trichoderma harzianum	43.9 (63.4)	54.8 (74.9)	51.3 (70.5)
Scytalidium FY strain	64.7 (82.8)	35.5 (54.3)	-

Table 4.1 Percentage inhibition of linear extension of *L. lepideus* 7F, 7B and *Scytalidium* FY strain produced by antibiotics of *Trichoderma* spp. and *Scytalidium* FY strain.

Figures in brackets indicate percentage inhibition based on colony area.

data and it is clear that hyphal extension and colony area of both *L. lepideus* strains are very significantly inhibited by presence of *Trichoderma* and *Scytalidium* produced antibiotics. Also evident from these results is the fact that growth of *Scytalidium* FY strain is also significantly inhibited by antibiotics produced by all three *Trichoderma* isolates.

Table 4.2 shows that the degree of growth inhibition of *L. lepideus* 7F induced by antibiotic substances of *T. harzianum* is substantially reduced due to the effect of autoclaving the culture filtrate prior to inoculation of the basidiomycete.

Inhibition of growth of *L. lepideus* 7F, by antibiotic compounds of Binab FYT pellet *Trichoderma* was not decreased by bubbling nitrogen gas N_2 through the filtrate prior to its use as a medium for the growth of the basidiomycete. This would suggest that the effect being monitored was not due to volatile products of the *Trichoderma*. Inhibition of linear extension and colony area of *L. lepideus* was marginally increased (linear extension from 54.8 → 63.6%, colony area from 74.9 → 81.7%) due to the bubbling of the gas through the filtrate.

4.3.2 Volatile antibiotic production

The technique used to detect volatile production clearly prevents hyphal and soluble metabolite contact

Target Fungus / *T. harzianum*	*L. lepideus* (FPRL 7F)
Filtrate heat treated	30.5 (46.8)
Filtrate not heat treated	54.8 (74.9)

Table 4.2 Percentage inhibition of linear extension of *L. lepideus* 7F produced by antibiotics present in both heat treated and non heat treated culture filtrate of *T. harzianum*.

Figures in brackets indicate inhibition based on colony area.

between interacting fungi and the results presented in Figure 4.3 and Plate 4.1 show that only the volatiles of *Trichoderma* spp. had any influence on the growth of *L. lepideus* irrespective of whether antagonists were placed above or below test fungi. Of the five basidiomycete species tested, *S. lacrimans*, *C. puteana*, *F. annosus* and *L. lepideus* showed varying susceptibility to the effects of volatiles from *Trichoderma* isolates. Only *Coriolus versicolor* appeared to be unaffected. Volatiles were produced at both 10° and 25°C although the slower growth rate of the organisms at the lower temperature makes the effect of the volatiles seem more acute (Table 4.3).

Scytalidium FY strain was markedly inhibited by volatiles from all three *Trichoderma* isolates, time for production of mean growth radii being increased from 214 hours for controls to 287, 338 and 713 hours by *T. polysporum*, *T. harzianum* and the Binab FYT pellet *Trichoderma* isolate respectively.

Table 4.4 shows percentage inhibition of hyphal extension of *L. lepideus* 7B, *L. lepideus* 7F and *F. annosus* when exposed to the three *Trichoderma* locations. As radial hyphal measurement was made in the direction of the location of each antagonist (three locations/plate) and five replicate plates were used, each value is a mean

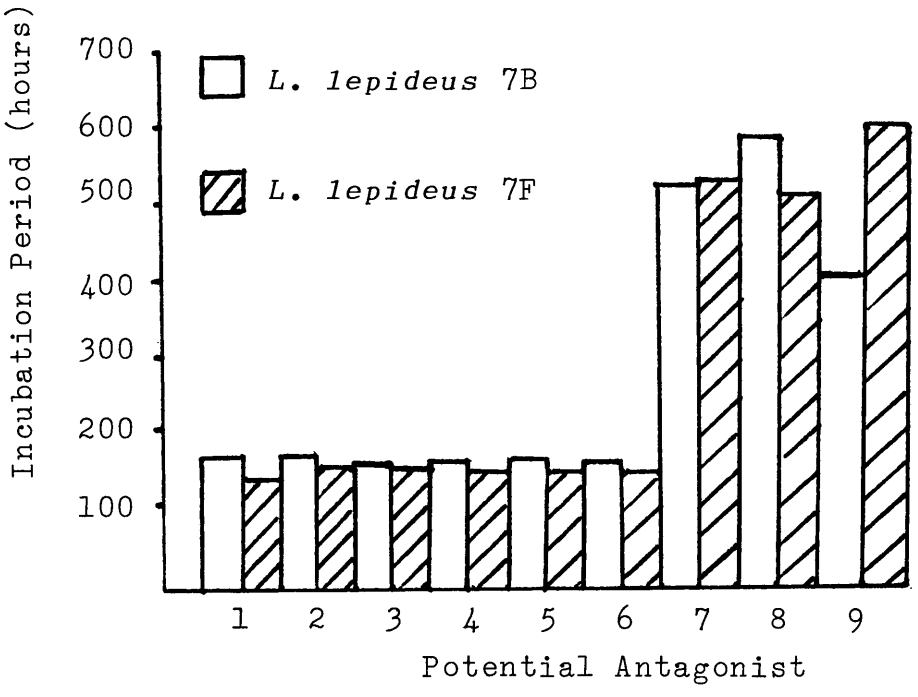


Figure 4.3 Incubation period (hours) required for *L. lepideus* cultures to produce radial growth of 35 mm when exposed to volatile metabolites of potentially antagonistic mould fungi
1) Control 2) *Aspergillus niger*, 3) *Penicillium chrysogenum*, 4) *Cladosporium resinae*, 5) *Fusarium* sp., 6) *Scytalidium* FY strain, 7) *Trichoderma* sp., 8) *Trichoderma harzianum*, 9) *Trichoderma polysporum*.

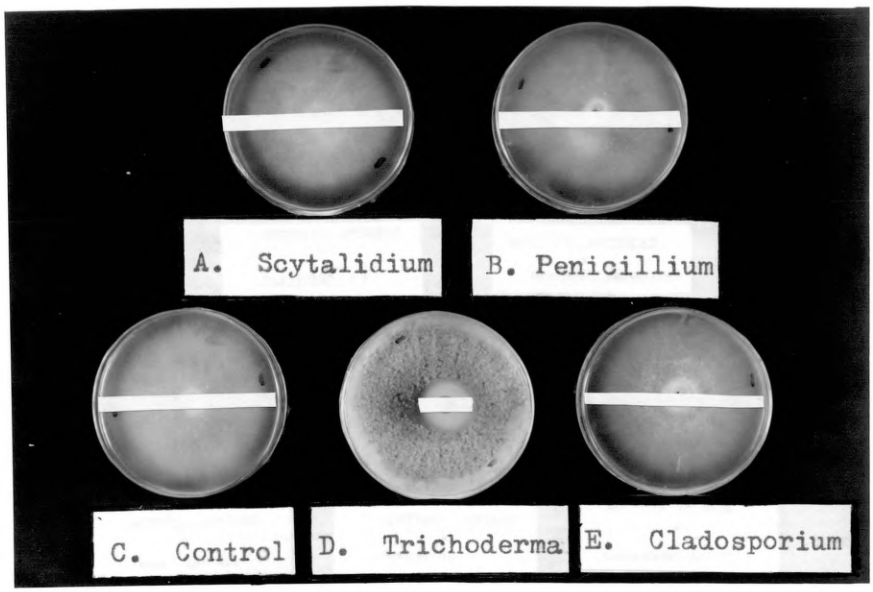


Plate 4.1 Growth of *L. lepideus* after incubation for 7 days when exposed in Dick and Hutchinson chambers to A) *Scytalidium* FY strain, B) *Penicillium chrysogenum*, C) control, D) *Trichoderma* spp., E) *Cladosporium resinae*. Growth of *Trichoderma* in the lower chamber is clearly visible through the uncolonised agar of the upper chamber in D. Limits of *L. lepideus* colonies are indicated by white lines on petri dishes.

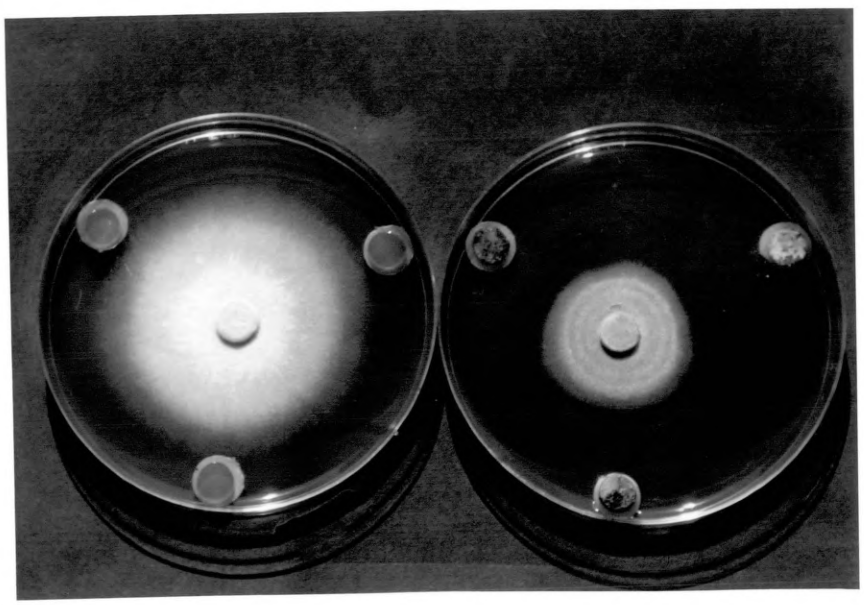


Plate 4.2 Growth of *L. lepideus* after 7 days when exposed to (left) uninoculated agar controls on plastic supports, (right) inocula of *Trichoderma* similarly located.

Temperature	10°C				25°C			
Antagonist / Target Fungus	Control	*Trichoderma polysporum*	*Trichoderma harzianum*	FYT pellet *Trichoderma* isolate	Control	*Trichoderma polysporum*	*Trichoderma harzianum*	FYT pellet *Trichoderma* isolate
L. lepideus (FPRL 7B)	1255	>1440	>1440	>1440	161	408	585	526
L. lepideus (FPRL 7F)	850	1440	>1056	>1032	141	334	470	648
F. annosus (FPRL 41E)	564	>2256	>2256	>1752	247	410	620	632
C. puteana (FPRL 11E)	612	812	1001	770	386	>552	>720	503
C. versicolor (FPRL 28A)	381	380	370	396	106	107	112	108
S. lacrymans (FPRL 12C)	1036	>2056	1715	>1592	666	1661	1567	816

Table 4.3 Time in hours for common basidiomycetes to grow 35 mm in the presence of volatiles produced by *Trichoderma* isolates at 10° and 25°C.

> symbol indicates that in some replicates the target fungus did not grow 35 mm prior to dehydration of the agar (about 90 days).

Target Fungus / Antagonist	*L. lepideus* (7B)	*L. lepideus* (7F)	*F. annosus* (41E)
Trichoderma sp.	22.7 (35.6)	37.1 (55.2)	83.1 (92.6)
T. polysporum	25.1 (38.9)	34.2 (51.6)	71.9 (85.5)
T. harzianum	20.3 (32.2)	29.9 (46.1)	88.7 (95.5)

Table 4.4 Percentage inhibition of linear extension of *L. lepideus* 7F, 7B and *F. annosus* 41E, when grown on agar plates, produced by volatiles of *Trichoderma* spp.

Figures in brackets indicate percentage inhibition based on colony area.

Distance from antagonist	10 mm	20 mm	30 mm
Percentage inhibition	52.6 (72.4)	49.6 (69.1)	25.2 (39.8)

Table 4.5 Effect of proximity of Binab *Trichoderma* locations on the percentage inhibition of linear extension of *L. lepideus* 7F.

Figures in brackets indicate percentage inhibition based on colony area.

of fifteen readings. Colony area was calculated from radial growth data and it is clear that hyphal extension and colony area are very significantly inhibited by *Trichoderma* presence (Plate 4.2). Table 4.5 shows that the degree of inhibition is a function of the proximity of antagonists to test organisms. Inhibition is obviously by volatile production as no contact with agar media supporting growth of basidiomycetes took place nor could take place due to location of antagonists on inert supports.

In a number of cases total inhibition of growth of the *L. lepideus* was obtained (Plate 4.3) and this was subsequently followed by the release of a red pigment into the agar. Microscopic examination of mycelium in the vicinity of the red pigmentation (Plate 4.4) shows that total disruption and cell wall lysis of *L. lepideus* mycelium had taken place. Sub-cultures removed from such inocula produced no growth of *L. lepideus* showing that the cultures were no longer viable and had been killed by volatile effects.

In plates in which only one antagonist core was placed, growth of the *L. lepideus* and *F. annosus* was reduced compared with controls and maximum growth inhibition occurred on the side of the test fungus nearest to the antagonist (Table 4.6 and Plate 4.5). The results also show that volatiles at high concentrations have fungicidal effects on *L. lepideus* while at low

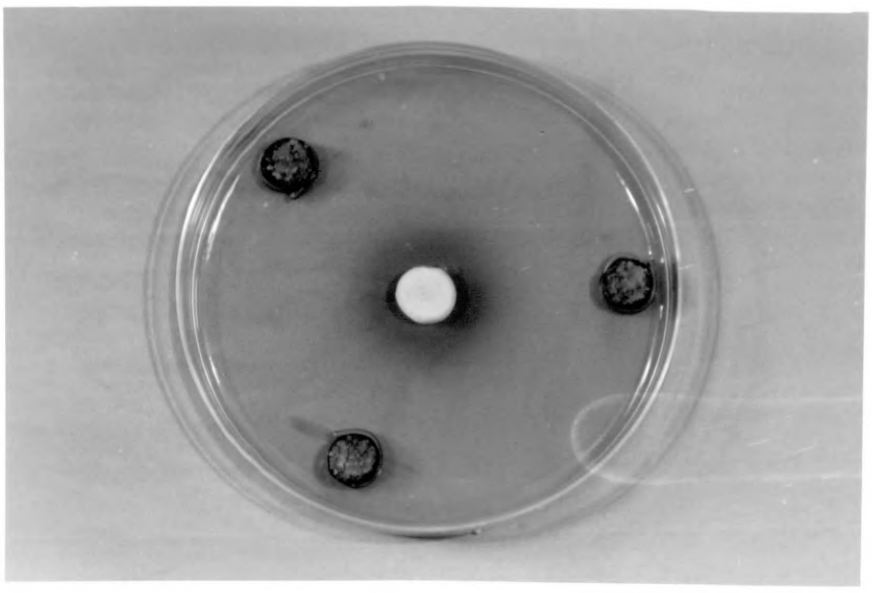


Plate 4.3 Complete inhibition of growth of *L. lepideus* by volatiles produced by *Trichoderma*. Darkening of media indicates initiation of autolysis in *L. lepideus* culture.

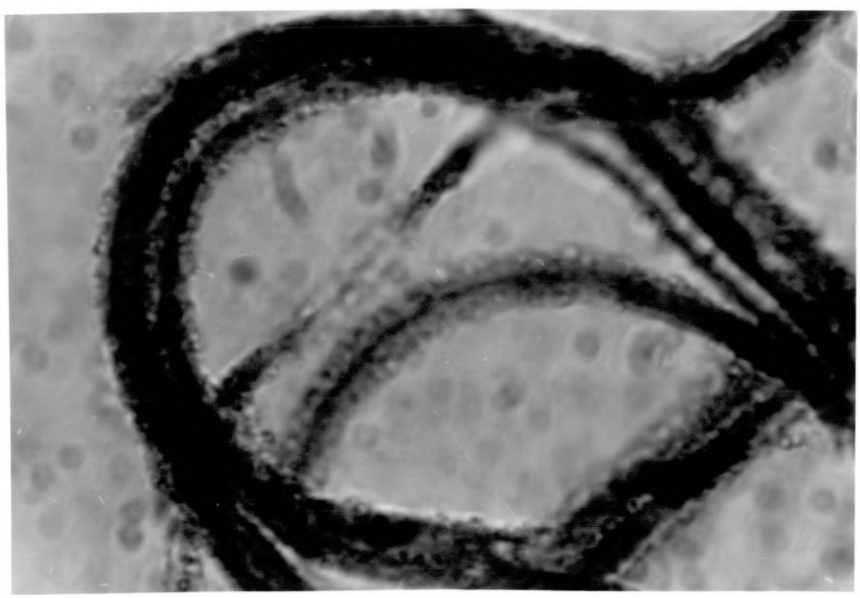


Plate 4.4 Photomicrograph of mycelium of *L. lepideus* in pigmented region showing cell disruption and cell wall breakdown.

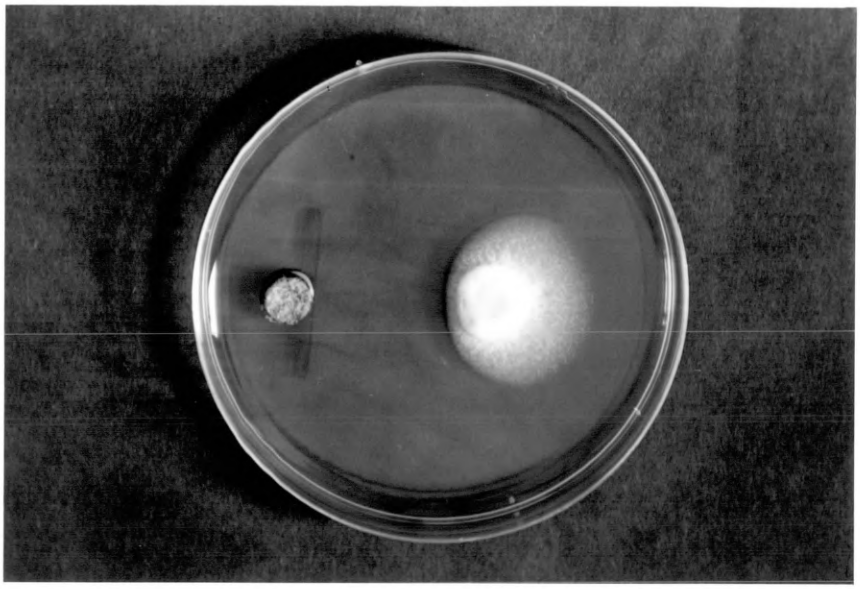


Plate 4.5 Directional response by *L. lepideus* to volatiles of *Trichoderma*. Lysis is taking place on side of colony closest to *Trichoderma* inoculum whereas on opposite side growth is virtually normal.

Target Fungus	*L. lepideus* 7B	*L. lepideus* 7F	*F. annosus* (41E)
Inhibition on side of colony nearest antagonist location	32.3%	86.3%	87.1%
Inhibition on side of colony furthest from antagonist location	0%	84.6%	49.3%

Table 4.6 Effect of sole antagonist location on the percentage inhibition of *L. lepideus* 7F, 7B and *F. annosus* produced by volatiles of Binab *Trichoderma* sp.

Pretreatment of wood blocks	Percentage inhibition
Trichoderma 1 month	26.4 (40.2)
Trichoderma 1 month, ethylene oxide sterilized	1.0 (1.7)
Trichoderma 1 month, ethylene oxide sterilized, leached	4.1 (6.8)
Trichoderma 1 month, autoclave sterilized	7.1 (11.8)

Table 4.7 Percentage inhibition of linear extension of *L. lepideus* (FPRL 7F) produced by volatiles from pine blocks pretreated with Binab *Trichoderma*.

Figures in brackets indicate percentage inhibition based on colony area.

concentrations fungistasis only is observed. This is particularly evident in Plate 4.5 which shows partial autolysis of *L. lepideus* indicated by darkening of the medium on the side of the colony closest to the antagonist whereas on the opposite side, at a greater distance from the antagonist, radial growth was normal.

When cores of control fungi were replaced with wood blocks pretreated with *Trichoderma*, inhibition of *L. lepideus* was achieved only in these blocks which contained live *Trichoderma* isolates (Table 4.7).

4.3.3 Identification of volatile components

Although good separation of the volatile components of culture gases of *Trichoderma* and *A. niger* was achieved with one column, no separation was obtained using the silicone oil packed column. This may have been due to incorrect column length, packing constituents or chromatograph operating parameters. The results obtained from the carbowax based column (Figure 4.4) show by retention values that *Trichoderma* and *A. niger* have almost the same components present in their culture gases however the quantity of the various constituents is different in the two cultures.

Of the six standards tested the retention times from the single column show that acetaldehyde, acetone and

Trichoderma culture gases

RT	AREA	TYPE	AR/HT	AREA%
0.52	1232700	PV	0.648	4.767
0.83	281970	VV	0.209	1.091
1.03	304240	VV	0.114	1.177
1.15	319590	VP	0.173	1.236
1.35	4701800	PV	0.178	18.184
2.29	5890900	VV	0.309	22.782
2.90	2411200	VV	0.179	9.325
3.02	2711600	VV	0.222	10.487
3.55	2252600	VB	0.297	8.712
4.59	1160800	BP	0.214	4.489
5.12	513790	PV	0.231	1.987
5.38	841080	VV	0.201	3.253
5.54	3235100	I VH	0.292	12.511

A. niger culture gases

RT	AREA	TYPE	AR/HT	AREA%
0.42	1283200	PV	0.591	2.633
1.11	406650	VV	0.267	0.834
1.24	511050	VV	0.115	1.049
1.35	336810	VP	0.157	0.691
1.95	6485300	PV	0.180	13.305
2.30	8896500	VV	0.329	18.252
2.91	8866200	VV	0.284	18.190
3.55	1.5141E+07	VB	0.200	31.063
4.61	3042800	BP	0.218	6.243
5.40	1610500	PV	0.223	3.304
5.57	2162600	I VH	0.199	4.437

STANDARDS

Isobutyl alcohol

RT	AREA	TYPE	AR/HT	AREA%
0.26	247580	BP	0.336	0.067
1.25	431880	PV	0.338	0.117
1.87	864100	VV	0.127	0.234
2.41	6777500	VV	0.185	1.833
3.00	3431900	VV	0.179	0.928
3.28	2063700	VV	0.180	0.558
3.51	502520	VV	0.153	0.136
3.84	2944600	VH	0.250	0.797
4.19	3.5177E+08	†SHH	0.681	95.151
5.66	663660	ITBB	0.312	0.180

Propionaldehyde

RT	AREA	TYPE	AR/HT	AREA%
1.05	137580	BV	0.066	0.662
1.25	63398	VV	0.100	0.305
1.89	910510	PP	0.130	4.380
2.37	1.2169E+07	PB	0.148	58.544
3.06	828020	BP	0.160	3.984
3.62	1298600	PP	0.209	6.247
4.31	836070	PV	0.221	4.022
4.71	4400800	VP	0.293	21.172
5.78	142270	I PP	0.339	0.684

N-butyraldehyde

RT	AREA	TYPE	AR/HT	AREA%
1.93	129070	PV	0.219	0.046
2.47	1058600	VH	0.210	0.378
2.79	2.7593E+08	†SHH	0.531	98.555
3.81	2857600	TBB	0.877	1.021

Acetone

RT	AREA	TYPE	AR/HT	AREA%
1.87	152070	BH	0.172	0.085
2.23	1.7865E+08	†SHH	0.344	99.617
3.50	534360	TBB	0.205	0.298

Ethyl acetate

RT	AREA	TYPE	AR/HT	AREA%
1.60	2.5518E+07	BV	0.763	11.271
2.32	1077100	VV	0.182	0.476
2.49	445110	VH	0.101	0.197
2.64	1.9725E+08	†SHB	0.381	87.121
3.56	831250	TBB	0.204	0.367
4.69	1230100	TBB	0.251	0.543
5.53	57545	I BP	0.357	0.025

Acetaldehyde

RT	AREA	TYPE	AR/HT	AREA%
0.98	56729	PV	0.072	0.057
1.08	71338	VB	0.102	0.072
1.79	5.9378E+07	ISBH	0.232	59.835
2.58	3119800	TBP	0.152	3.144
2.98	7195700	TPV	0.255	7.251
3.27	701220	TVV	0.173	0.707
3.51	1077000	TVP	0.266	1.085
5.54	2.7636E+07	ITPB	0.348	27.849

Figure 4.4 Gas chromatographs of culture gases and standards. RT-Retention time. Area % - percentage proportion of each constituent.

propionaldehyde appear to be among the major constituents. The accuracy of these findings however is limited since only one column was used and thus no reinforcement data is available and multiple peaks were produced during analysis of the standards which should not be the case when pure compounds are used. The results do however show that one of the major constituent gases produced by both *Trichoderma* and *A. niger* with a retention time of 3.55 minutes was not among the standards which were tested.

4.4 DISCUSSION

The results of Chapter 2 established that *Trichoderma* could overgrow and produce lysis of *Lentinus lepideus* mycelium when grown together in agar culture and the wood block studies in Chapter 3 have shown that *Trichoderma* residues are capable of protecting wood blocks from decay by that fungus, although this protection appeared to be lost after autoclaving of the wood. The experimental designs and observations made in these two chapters have shown that hyphal interaction and competition for nutrients, usually considered to be major sources of control exerted by *Trichoderma*, were not responsible for the control of *L. lepideus*. The results of the experiments described in this chapter show that *Trichoderma* spp. produce heat labile antibiotics effective against *L. lepideus* and also

that volatiles alone produced by *Trichoderma* were responsible for inhibition and lysis of the decay fungus.

The results showing antibiotic production by *T. polysporum*, *T. harzianum* and the mixed *Trichoderma* culture agree with the findings of Dennis and Webster (1971a) and Park and Robinson (1964) that *Trichoderma* spp. produce soluble substances with antifungal qualities. Dennis and Webster (1971a) showed that various *Trichoderma* spp. could produce antibiotics active against a range of fungal classes including the basidiomycete *F. annosus*. Their results indicated that of 14 isolates of *T. polysporum* tested 13 were shown to be antibiotic producers and of 9 isolates of *T. harzianum* tested 6 were shown to produce antibiotics 3 of which were effective only against the decay fungus *F. annosus*. The *T. polysporum* and *T. harzianum* strains used in this current study produced antibiotics active against both *L. lepideus* and *Scytalidium* FY strain. Whether the antibiotics active against *L. lepideus* are Trichodermin, a chloroform soluble antibiotic reported to be produced by *T. viride* (Godtfredsen and Vangedal, 1965), is open to debate, but it is thought unlikely since the results of Dennis and Webster (1971a) show that of five isolates of *T. polysporum* and *T. harzianum* tested only one produced Trichodermin. An alternative theory would be that the antibiotics produced

by all the *Trichoderma* strains used in this study, although different from Trichodermin, may be identical to the fungal ageing hormone shown by Park and Robinson (1964) to be produced by *Trichoderma viride*.

The ability of *Trichoderma* spp. to produce volatiles capable of exerting fungistatic and fungicidal effects on *L. lepideus* did not appear to be shared with any of the other common moulds normally associated with creosoted wood. *Fusarium* and *Penicillium* were shown to be capable of overgrowing *L. lepideus* when grown together in the same agar plates and such overgrowth is always accompanied by mycelial lysis in the target organisms. In view of the lack of inhibiting volatile production by the former it is probable that such early lysis may be attributed to soluble metabolite production.

Inhibiting or lethal effects of *Trichoderma* volatiles are apparently wide spectrum amongst basidiomycetes although the extent and severity of the volatiles varies largely between species. The only basidiomycete tested upon which the volatiles had no apparent effect was *Coriolus versicolor* and significantly this was the only fungus which was not replaced by *Trichoderma* during the cross plating reactions studied earlier. This result stresses the possible importance which volatile production may play during fungal succession.

Previous work carried out to determine fungal production of volatiles and their effects on the growth and spore germination of other fungal species (Dick and Hutchinson, 1966; Glen and Hutchinson, 1969, 1973; Glen, Hutchinson and McCorkindale, 1966; Robinson and Park, 1966; Robinson and Garrett, 1969; Robinson, Park and Garrett, 1968; Dennis and Webster, 1971b; Tamini and Hutchinson, 1975) showed only fungistatic and not fungicidal effects although Russian workers Bilai (1956) and Kashonov (1962) (quoted in Dennis and Webster, 1971b), have reported production of fungicidal volatiles by some *Trichoderma* isolates. The results presented here support the Russian observation and it would seem that the volatiles of *Trichoderma* spp. may lethally effect *L. lepideus*. It is possible that a similar phenomenon may also explain the lack of colonisation of oak bed logs by the edible shiitake mushroom *Lentinus edodes* when pre-exposed to *Trichoderma*.

Although the attempts which were made to identify the chemical constituents of the volatiles produced by *Trichoderma* were limited in scope it was felt that with further research the process of gas collection and gas chromatography could be extended to enable identification and quantification of the volatile constituents. The results however do show that all the major constituents of the volatile fractions collected from both *Trichoderma*

and *A. niger* are common to both even although their relative proportions differ.

It was noticeable that levels of acetaldehyde were consistently higher in the *Trichoderma* fractions compared to the *A. niger*. The presence of this compound confirms the findings of Dennis and Webster (1971b) who isolated this chemical in culture gases of various *Trichoderma* isolates and showed that aqueous solutions of acetaldehyde produced fungistasis in *F. annosus* identical to that produced by volatiles from *Trichoderma*. These authors also showed that a 100 ppm solution of acetaldehyde caused slight inhibition of growth in *F. annosus* while 500 ppm and above caused complete inhibition. Comparison of the chemical components present in volatiles collected from cultures of *Trichoderma* and *A. niger* showed that the presence of acetaldehyde alone did not produce inhibition of *L. lepideus* however high concentrations of that chemical may be responsible for the fungistatic and fungicidal effects observed. Robinson and Park (1966), Robinson, Park and Garrett (1968) and Robinson and Garrett (1969) indicated that acetaldehyde accounted largely for the vapour phase sporostasis of several fungi with reported levels of 15 ppm causing appreciable retardation of spore germination of *Cunninghamella elegans*.

Unless a specific component, present in undetectable quantities, produced the inhibitory effects of *Trichoderma* against *L. lepideus* it would appear that concentration effects of common volatile components were responsible for the volatile activity of *Trichoderma*.

Further gas chromatography is required however to determine if acetaldehyde is solely responsible for the fungistatic and fungicidal effects of *Trichoderma*. As suggested by Dennis and Webster (1971b) it is more likely that other volatile metabolites acting singly or in combination are also involved. Robinson and Garrett (1969) have done an extensive study on volatile components from *Fusarium oxysporum* which included acetone, acetaldehyde and propionaldehyde. Their results showed that complex interactions occurred between individual components which determined the overall volatile activity of the fungus. It is likely that a similar phenomenon exists in *Trichoderma* and further gas chromatography is warranted in order to investigate fully this area of study.

The effects described in this chapter are largely the results of agar studies and may not thus be extrapolated directly to the field. However fungal mycelia in wood occupy enclosed spaces and are very proximate to each other when occupying individual elements e.g. fungal mycelia measure 2-10μ in diameter

and lumina of fibres and tracheids range from 10-20μ. It is not unreasonable to suggest that under such circumstances inhibitory volatiles produced by one occupant of an element may have very significant effects on others and practical experience shows that except under exceptional circumstances, e.g. creosoted wood, timber contains many different microbial populations. Volatile production by fungi in microbial succession may thus determine patterns of further microbial invasion especially in the short term or at least until such times as nutrients are depleted and volatile production ceases. Short term biological control by competition for available nutrients to protect wood from post harvest deterioration as proposed by Hulme and Shields (1970) may thus be enhanced by the ability of the control agents to produce volatiles.

Inhibitory volatiles produced by fungi may also have significant longer term practical applications if these are lethal and can be applied to appropriate target organisms. If *Trichoderma* can be applied to creosoted timber in the field and at the same time retain the ability to produce both non leachable antibiotics as well as volatiles and, as *L. lepideus* is the major cause of decay of creosoted wood, with appropriate strain selection *Trichoderma* might well be a suitable control agent for this specific circumstance. However, Dennis

and Webster (1971a,b) noted large interstrain variability in antibiotic and volatile production in the genus *Trichoderma*, thus much variation in effects on target organisms might be expected from casual usage of randomly selected strains.

One significant fact to emerge from the results of this chapter is the incompatability of the *Trichoderma* isolated from Binab FYT pellets and *Scytalidium* FY strain. The *Trichoderma* isolate produced both antibiotics and volatiles which were effective in inhibiting the growth of *Scytalidium* FY strain and this may explain why *Scytalidium* has never been isolated from cultures grown from Binab FYT pellets. This result implies that any biological treatment combining both *Trichoderma* spp and *Scytalidium* (such as the Binab FYT pellet) might be ineffective as growth of only the *Trichoderma* will take place, at least in the short term, and growth of *Scytalidium* FY strain would be suppressed in the early stages.

CHAPTER 5

MOISTURE AND NUTRIENT LEVELS OF CREOSOTED POLES IN SERVICE

5.1 INTRODUCTION

An investigation carried out by the Midlands Electricity Board (Anonymous, 1971) indicated that creosoted distribution poles have an average service life of 35 years. This study also showed that the greatest incidence of decay occurred in the region of one foot above or below ground level and that decay was most predominant in a band between the heartwood and a point ½" below the pole surface. The investigators concluded that such internal rot was responsible for nearly all pole failures and that rot elsewhere was relatively insignificant and could be disregarded. *Lentinus lepideus* has long been accepted as the basidiomycete responsible for decay of creosoted timbers (Cartwright and Findlay, 1958) and King and Penn (1975, unpublished data) identified that organism as the major causative organism of internal decay of creosoted distribution poles in the Midlands region.

While little work has been undertaken to examine the factors which influence internal decay of creosoted distribution poles it is generally considered that the incidence of decay produced by *L. lepideus* in poles is affected by physical and environmental parameters which

include, moisture content, nutrient status, pH, temperature, oxygen availability, geographic location, wood type, extent of creosote penetration, presence of any remedial 'cobra' treatment, competition from resident non-decay fungi and presence of cracks and splits in the timber. This last mentioned parameter is most important since it is through these avenues that *L. lepideus* spores gain entry past the creosote treated protective sheath into the unprotected interior, especially if poles are inadequately treated.

5.1.1 Moisture

Moisture levels and the closely related oxygen availability are most important in determining whether aerobic organisms can grow, develop and reproduce efficiently in creosoted poles. Since most decay occurs in the groundline region of poles it is at these regions that the moisture content of the poles is critical for decay to develop and as it is into these regions which biological control organisms will be inoculated, their development will also be dependent on these moisture levels.

The Midlands Electricity Board have developed a specialised elongated probe which when used as an attachment to a conductivity meter measures the moisture contents of pole interiors. Baines and Levy (1979) have

suggested that moisture is not evenly distributed in wood in soil and that cones of moisture are produced in the groundline region of such timber. If such cones of moisture do exist in creosoted poles they may lead to incorrect readings on the moisture meter. These may occur if the two points across which the conductivity reading is measured are positioned either across the boundary of the core of moisture or indeed are totally outside the high moisture region (Figure 5.1).

Plate 5.1 The use of the Protimeter and specially developed probe to measure moisture contents of poles.

There is also presently much debate as to whether moisture gradients found in wooden distribution poles in soil contact are due to wick action as suggested by Baines and Levy

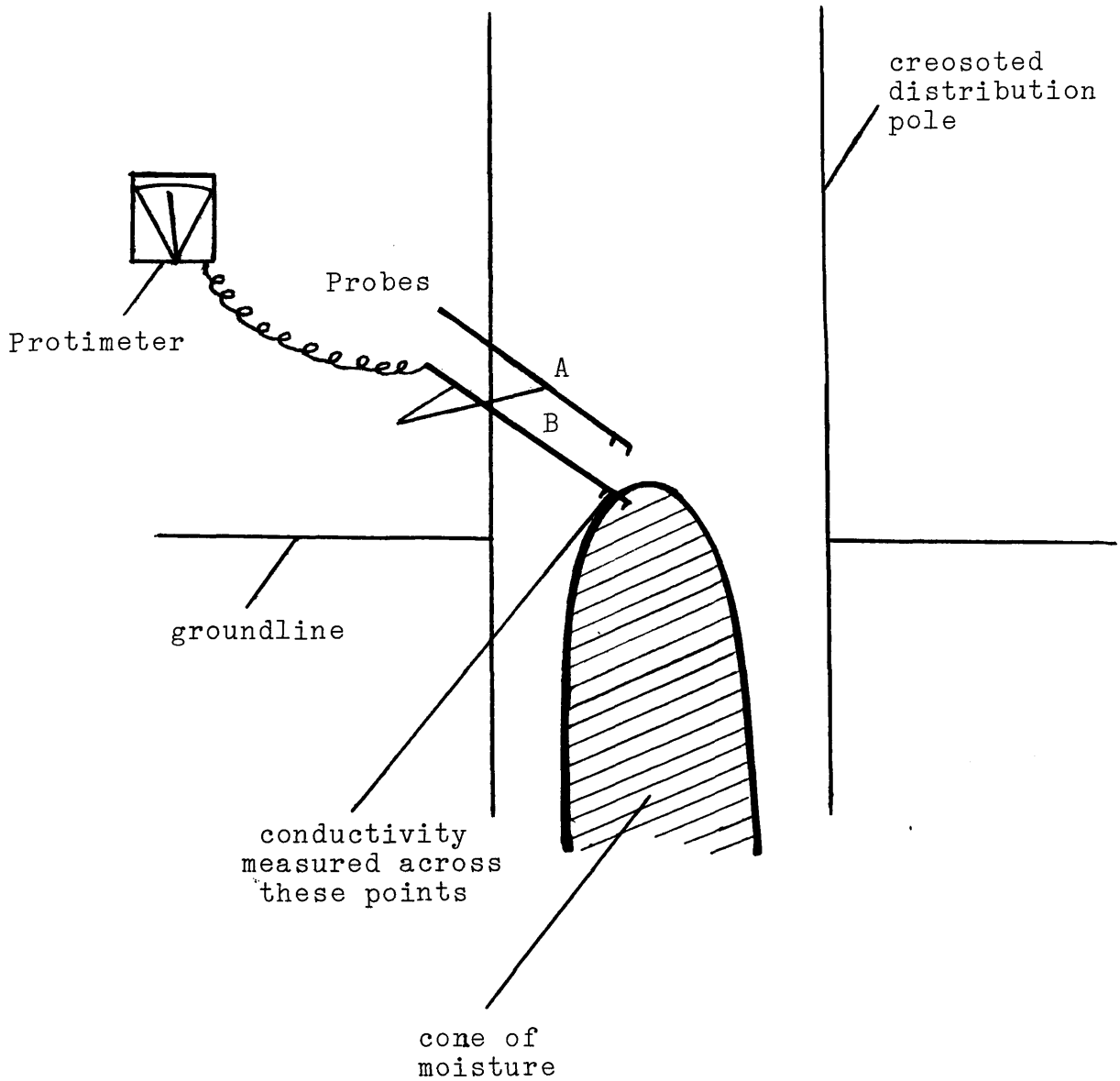


Figure 5.1 Possible sources of error with use of Protimeter for moisture measurement.

(A - Probe outwith wet region)

(B - Probe placed across boundary of cone of moisture)

(1979) or whether they are due to rainwater running down through the fibres of the poles or if indeed both mechanisms are involved.

5.1.2 Nutrients

Wood, even when treated with some types of preservatives has been shown (Mowe, King and Senn, 1983) to act as an attractant to certain soft rot fungi and so it is inevitable that decay will eventually take place. These authors also showed that wood treated with creosote acted as a repellant. King and Waite (1979) postulated that nitrogen levels in wood increased only as part of the decay process via translocation by pioneer colonisers of the wood. Since transmission poles are externally creosote treated nitrogen will not be transported in this fashion into the wood. Uju, Baines and Levy (1981) however have proposed that nutrient levels of wood in soil increase via the uptake of soluble nitrogen salts by wick action. This observation is supported by a common belief held among the electrical authorities that poles situated in rich agricultural land are more susceptible to decay than those in uncultivated sites.

Wood decay can take place without any great increase in nitrogen content by the action of basidiomycetes (Klingstrom and Oksbjerg,1963) as these organisms do not

require high levels of nitrogen before they can produce the enzymes responsible for decay. However the presence of excess nitrogen stimulates even higher rates of decay. Biological control fungi including Binab FYT organisms *Trichoderma* and *Scytalidium* however do not produce decay enzymes (a capability which would make them worthless as control agents). Therefore unless there is nutrient transfer from soil to creosoted poles during moisture uptake by wick action, the only source of nutrients readily available for consumption by control fungi are those left in the ray tissues of the poles during curing of the timber and those present in the Binab FYT pellets. Possible transfer of nutrients may therefore determine both the duration of viability and possible biomass production of control organisms in creosoted poles.

The aims of the following field and laboratory work were therefore,

1) To establish the range of moisture contents present in poles in service in the field.

2) To establish the extent to which nitrogen transfer occurs from soils into pole interiors.

3) To examine whether any intersite variation in pole nitrogen contents exists.

5.2 MATERIALS AND METHODS

5.2.1 Moisture

Two hundred and sixteen distribution poles in service at three sites, respectively, Glenlethnot and Inchture in Scotland and Ludlow in the West Midlands of England (inoculated with FYT pellets as described in detail in Chapter 6) were regularly sampled for moisture content over a 30 month period. Each of the sites had a different soil type. At Glenlethnot the soil was peat based with a high organic content beneath course heather moorland and is unlikely to ever have been under cultivation. The Inchture site consisted of good agricultural land used regularly for fruit and cereal growing, the soil was a rich loam which had received regular fertilizer application. The soil at Ludlow was clay based and the land is used mainly for cattle and sheep grazing although in the recent past some may have been cultivated for cereal crops and received some fertilizer treatment.

Moisture contents of the poles were measured using a "Protimeter" moisture meter and the elongated probe developed by the Midlands Electricity Board (Plate 5.1). A flame sterilized Mattson augur was drilled into each pole at a point 10 cms above groundline at an angle of 30° to the horizontal thereby ensuring that the borer

reached the centre of the pole at approximately the groundline. The wood core thus produced was carefully extracted and the moisture meter probe fully inserted into the bore hole. The probe was held tightly into the pole while the moisture reading was recorded to ensure good contact between the two points of the probe and the interior surface of the pole. Poles were sampled at the time of inoculation with FYT pellets and thereafter at intervals of initially 3 and then 6 months over a period of 30 months primarily to monitor the growth of the biological control organisms. However during these sampling periods the moisture contents of the poles were also measured. In general, moisture contents were measured at points located at 3 cm increments above the groundline to a height of 15 cms. The "Protimeter" operated by measuring electrical conductivity of the wood and recorded the moisture contents of a range of wood types on a scale from 0-30%. Poles, the moisture contents of which were higher than 30%, were recorded as either 30+ or 30++ depending how far off the scale the "Protimeter" needle moved. 30+ represented a moisture content of between 30 and 33%.

Two poles 35 feet in length and sited 20 feet apart at the Linesman Training School at Ludlow were selected for sampling in an attempt to monitor moisture distribution in poles. The two poles were identical with regard to

their length, position, creosote status and the depth to which they were embedded and differed only with respect to their date of erection. Pole 1 had been in position for approximately 3 years while pole 2 had been erected only 3 months previously. Cores of wood were removed using a Mattson hand driver augur every three to four feet along the length of the poles from groundline to top (Plate 5.2). The moisture contents at each of these boreholes were then measured using the Protimeter and probe.

Plate 5.2 Wood cores being removed along the length of a creosote treated pole to enable moisture levels to be measured.

5.2.2 Nutrients

A total of 204 poles were sampled for nutrient content, 81 mature poles at Glenlethnot, 33 similar poles at Inchture and 90 poles at Ludlow of which approximately 50% were mature (Mature poles are those which have been creosote treated and on site for at least 25 years). The cores removed from these poles during routine sampling, consisted of a creosote treated outer portion, an untreated sapwood region and an untreated heartwood section. On return to the laboratory the creosoted portion was removed and the remaining core halved. The sapwood containing portion was plated onto agar to facilitate the isolation of organisms resident in poles while the other half of the core was used for nitrogen analysis. Nitrogen content was determined by a micro-Kjeldahl technique (Humphries, 1956) in which hydrogen peroxide was used as a catalyst (Bruce, 1980). Nitrogen analysis was also carried out on wood cores which were removed from 6 copper-chrome-arsenate treated poles in Sweden by the Swedish Department of Forest Products. These cores were removed 10 cms above groundline from poles which had been on site for between 8 and 11 years. All the poles were sound and situated in differing soil types under different moisture conditions.

Ten soil samples were also collected from each of the three sites. These samples each consisting of about

30 grams were removed from around the bases of poles at a depth of about 10 inches. Collected samples were placed in polythene bags which were stored in a fridge before nitrogen analysis. Soil samples were oven dried at 40°C for 1 week prior to nitrogen determination. Large pieces of organic debris and stones were removed before the soils were passed through a 2mm sieve. These samples were then ground using a mortar and pestle and these ground samples were split into two portions one of which was used for nitrogen analysis while the other was used to calculate the moisture content of the partially dried soil. As for the wood cores, the micro-Kjeldahl technique (Humphries, 1956) was used for soil nitrogen analysis. In contrast to the analysis of the wood however great care was required when adding the hydrogen peroxide to the acid digest of the soil due to the vigour of the ensuing reaction.

Three replicate analyses were carried out on each soil sample. The results from the ten samples taken from each site were then used to calculate a mean value for soil nitrogen in each region.

5.3 RESULTS

5.3.1 Moisture in wood

Moisture contents of creosote treated poles at Glenlethnot, Inchture and Ludlow are presented in Figures 5.2,

5.3 and 5.4 respectively. These show that moisture contents of poles varied from 18% in dry poles to poles which were virtually standing in water and completely saturated. This variation occurred not only between sites but also between poles in the one area depending largely on the microenvironment of the individual pole. Little seasonal variation in the moisture contents of individual poles was apparent although Figures 5.2 - 5.4 show a slight shift towards higher moisture levels over the winter period of October → March at all sites.

Since marginal climatic differences exist between the three sites as shown by their mean annual rainfall figures of

* Glenlethnot - 39.7"
* Inchture - 31.1"
* Ludlow - 28.9"

the percentage of poles at each site with moisture contents of greater than 30% were calculated at each sampling period. In order to minimise the seasonal variation, mean figures were calculated from the values at each sampling. These figures, representative of the high moisture content poles at each site, were then plotted against the average mean annual rainfall for each site and

* The atlas of Britain and Northern Ireland Claredon Press - Oxford 1963

Figure 5.2 Moisture contents of creosoted poles at Glenlethnot.

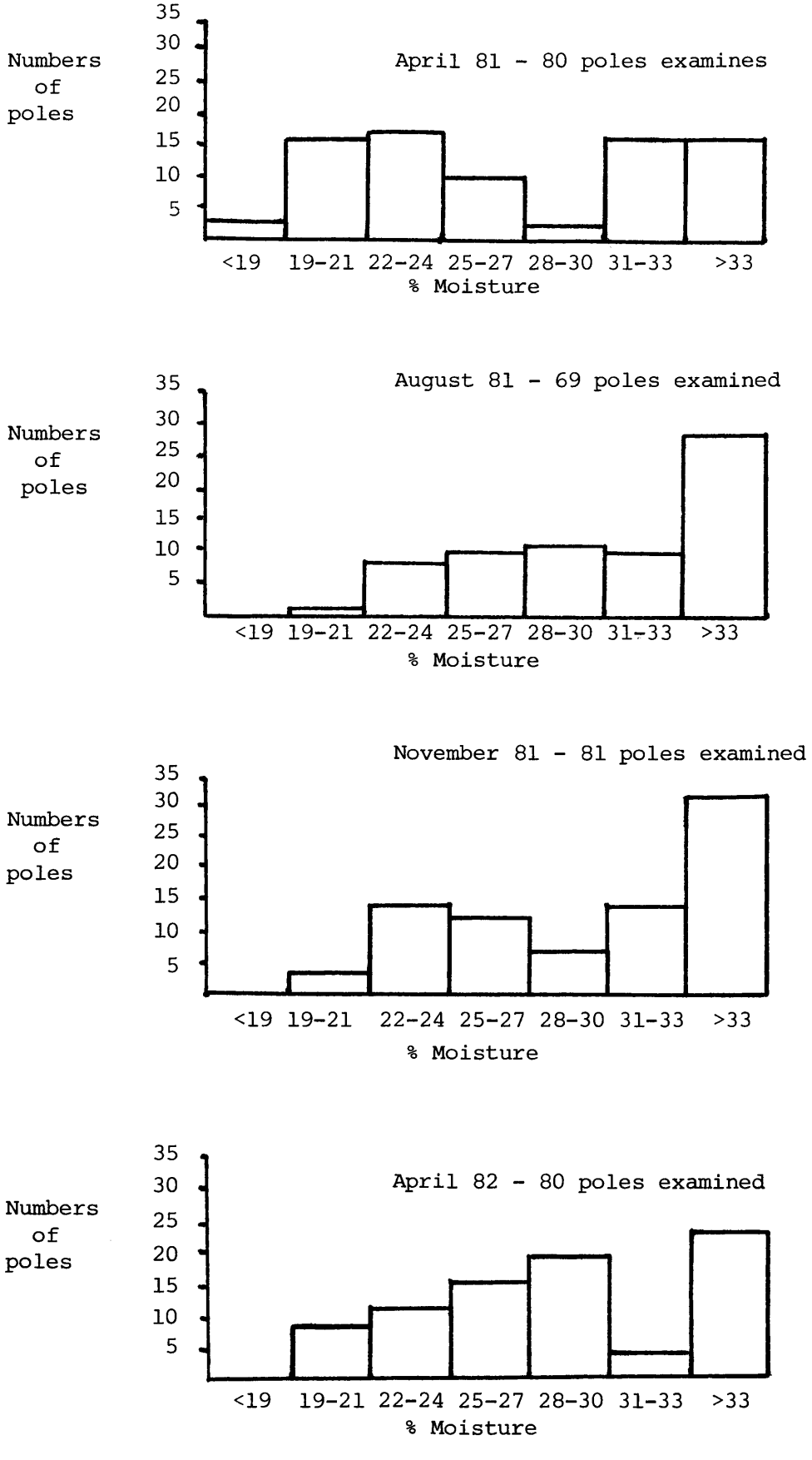


Figure 5.2 (contd.) Moisture content of creosoted poles at Glenlethnot

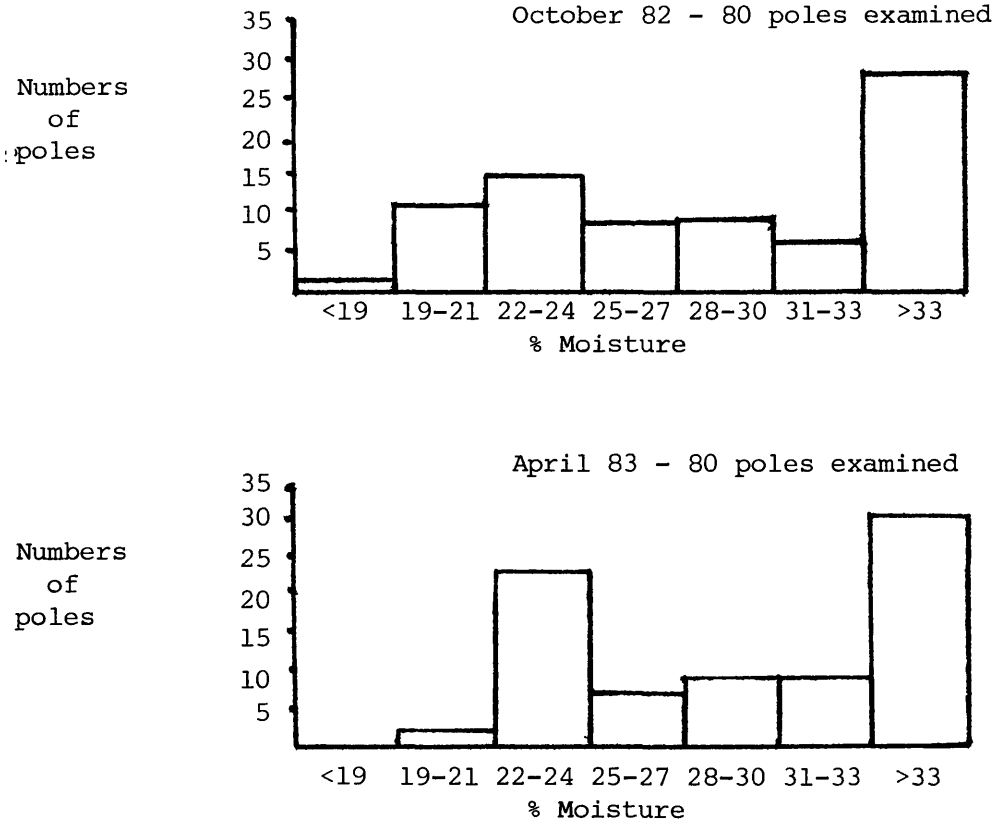


Figure 5.3 Moisture contents of creosoted poles at Inchture.

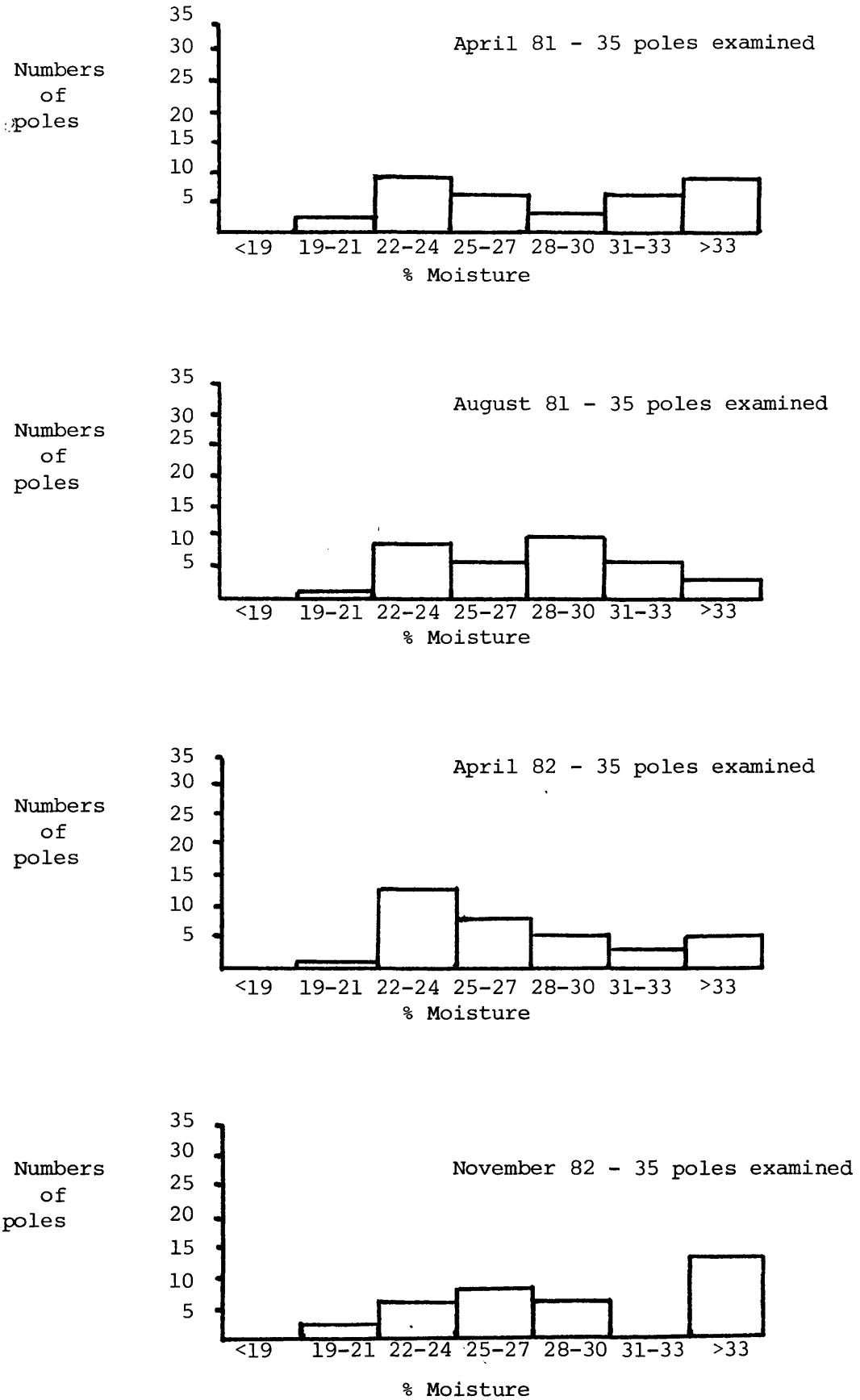


Figure 5.3 (Contd.) Moisture contents of creosoted poles at Inchture.

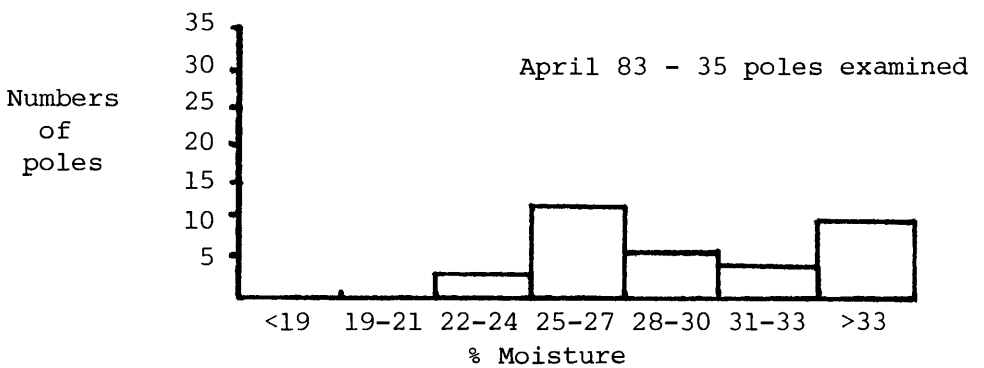


Figure 5.4 Moisture contents of creosoted poles at Ludlow.

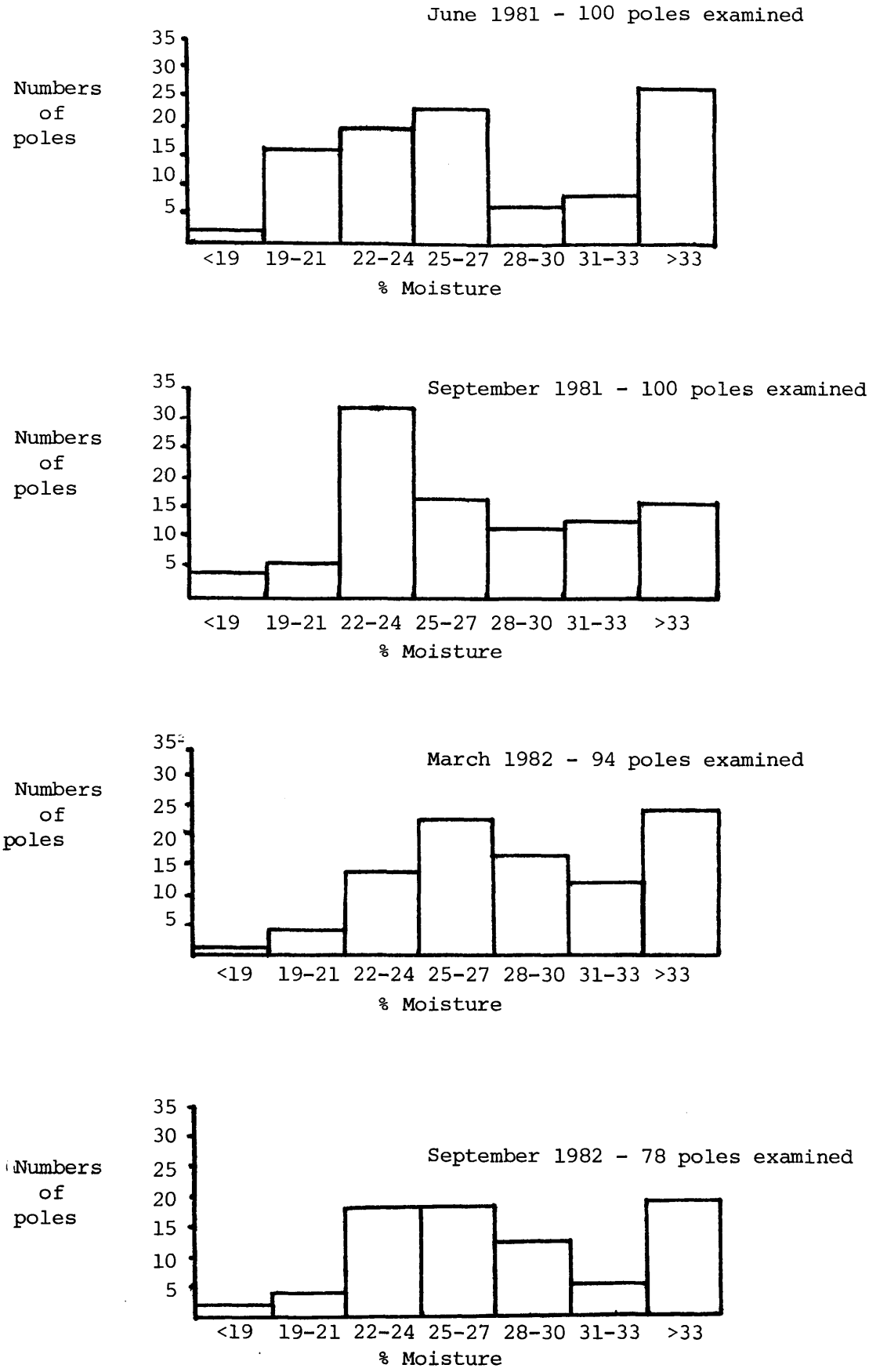
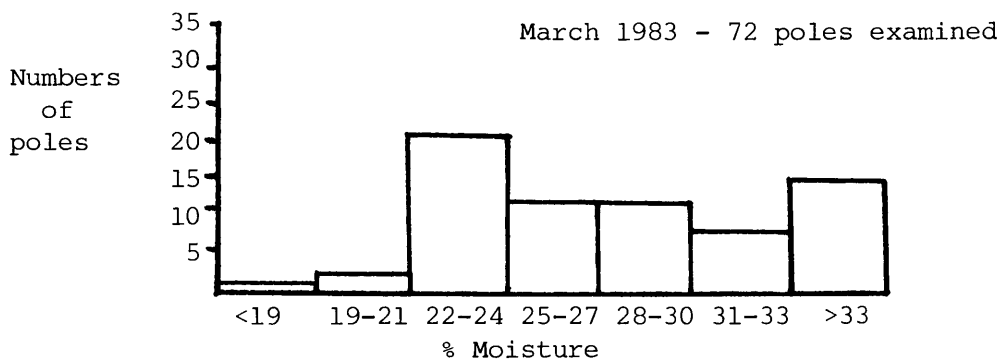


Figure 5.4 (Contd.) Moisture content of creosoted poles at Ludlow.



the results (Figure 5.5) show that there is a direct relationship between mean annual rainfall and the moisture contents of poles.

Table 5.1 shows the moisture contents at various positions along the length of two creosote treated distribution poles. These indicate that pole 1 which had been on site for three years contained a moisture gradient from pole top to groundline while pole 2 erected only three months before the sampling date had a moisture gradient over only the 10 feet immediately above groundline.

5.3.2 Nitrogen Transfer

The results for nitrogen content of creosote treated poles and surrounding soils both in Britain and Sweden are shown in Tables 5.2 and 5.3 respectively. These show that although the soil nitrogen values for the three sites in Britain are widely different the mean pole nitrogen contents at these sites are almost identical and are similar to those found in the Swedish poles.

5.4 DISCUSSION

The results presented here show that moisture contents of creosoted distribution poles in service range

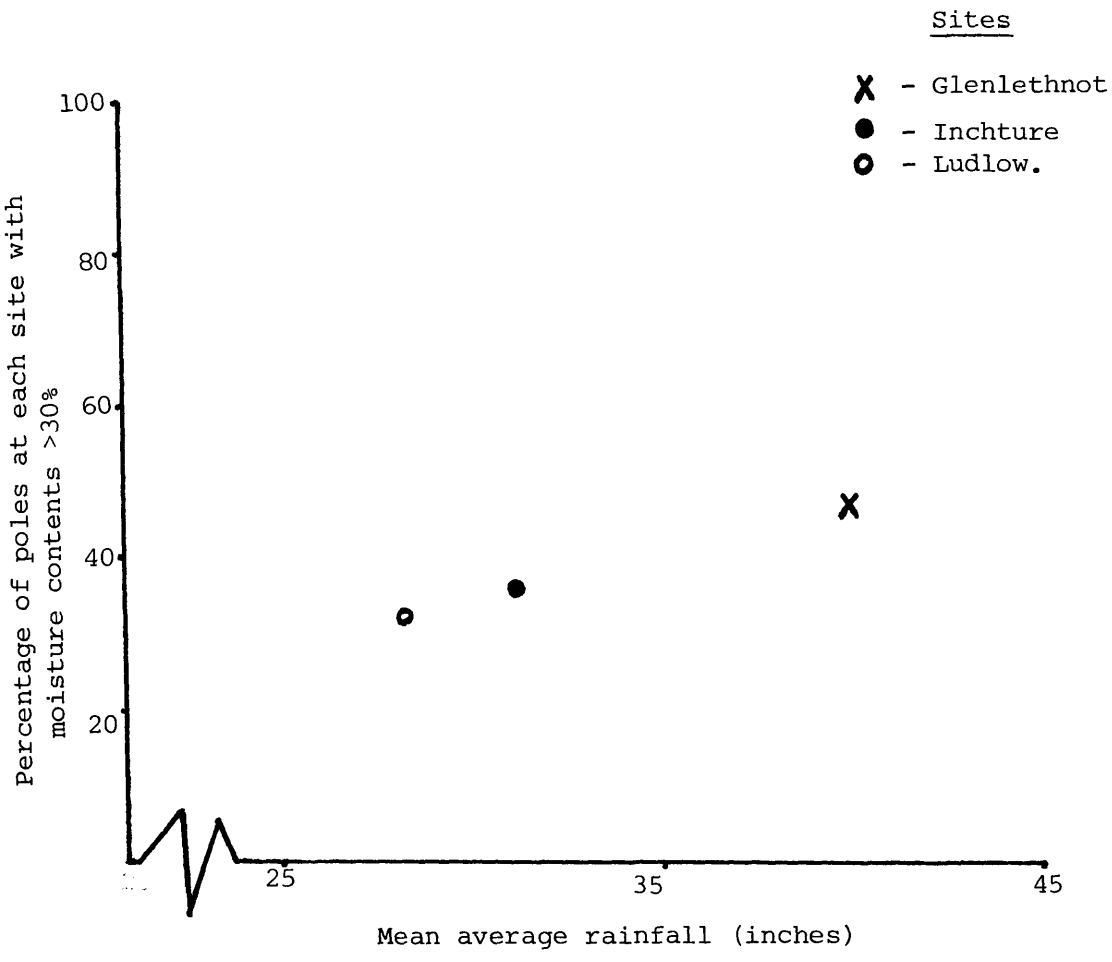


Figure 5.5 Shows the direct relationship between mean average rainfall and moisture content of transmission poles at the three geographical sites.

Height from Ground (ft)	Pole 1 Year of erection - 1979	Pole 2 Year of erection - 1982
34.0	13.5	19.0
32.0	13.5	22.0
29.0	16.0	22.5
24.0	-	19.5
21.0	16.0	19.5
18.0	16.0	-
15.0	17.0	17.0
11.5	17.5	-
10.0	-	16.0
6.5	21.5	17.5
4.0	23.0	19.0
2.0	26.0	21.0
1.0	30.0	22.0
0	30.0	22.0

Table 5.1 Moisture contents at various positions along the length of two creosote treated transmission poles.

	Percentage nitrogen	
Location	Soil	Poles
Inchture, Perthshire	0.121 ± 0.06	0.063 ± 0.0035
Glenlethnot, Forfarshire	0.385 ± 0.15	0.062 ± 0.0023
Ludlow, Shropshire	0.127 ± 0.07	0.056 ± 0.0021

Table 5.2 Nitrogen content of soil at three field sites in Great Britain. The nitrogen contents of uncreosoted interiors of poles in service in these soils for periods in excess of 25 years are also presented.

Wood Type	Site	Nitrogen content
Pine	Wild pasture, no fertilizer	0.051
Pine	Cultivated pasture, fertilized	0.043
Pine	Ash forest, wet	0.045
Pine	Ash forest, wet	0.071
Pine	Beech forest, well drained	0.033
Beech	Test Site. Dept. Forest Products, Uppsala	0.100

Table 5.3 Mean nitrogen contents of wood samples removed from 10 cm above groundline in C.C.A. treated poles in Scandinavia.

from 18% - >33% with the occasional pole being totally waterlogged. Approximately 35% contained moisture contents of higher than the fibre saturation point. Moisture content and distribution within poles will have a significant effect on the success of any biological control system as it will determine the proportion of poles into which the control agents can become established. In the same manner moisture content will determine the frequency of *L. lepideus* infection. The ability of a control agent to grow and develop over a wide range of moisture contents would therefore appear to be essential if it is to successfully control decay in creosoted poles. *Trichoderma* species are natural colonisers of freshly felled timber and would thus be expected to be able to colonise wet wood without problem, however their ability to colonise wood with moisture contents as low as 18% must be in doubt.

Many techniques have been employed to measure decay in wood in the field including X-ray, ultrasonic and wood boring techniques, however few have been manufactured for measuring moisture contents. Ordinarily moisture contents of wood are calculated by weighing, oven drying and reweighing samples in the laboratory. However this process is not practical for use on samples removed from creosoted poles since moisture levels may change during transport from field site to the laboratory and moisture

may be lost due to compression factors involved during removal of wood cores. Electrical conductivity meters, e.g. the "Protimeter" moisture meter used during this study, have been manufactured to monitor moisture contents of wood in the field and operate by measuring the electrical conductivity between two probes inserted into wood. If moisture is not evenly distributed in the wood as suggested by Baines and Levy (1979) problems can arise with the use of such meters, due to the positioning of the two points across which the conductivity is measured (Figure 5.1). The results however show that even although poles were sampled at different heights on subsequent sampling visits, those poles which gave high moisture contents at the first sampling had consistently high moisture contents throughout the sampling period. Because of the differing sampling heights there is no possibility that in every case the measurement was recorded across the boundary of the cone of moisture should it exist, and so the results for pole moisture contents can be accepted as being accurate for the groundline regions of the poles. 'Cobra' treated poles may also present problems for the use of such moisture meters since the salts left in the wood may effect conductivity readings. Preliminary experiments at this laboratory however, have shown the "Protimeter" and probe to be accurate to within 1% for untreated wood and to

within 2% for 'cobra' treated poles. These were carried out by weighing, oven drying and reweighing samples removed from untreated and 'cobra' treated pole sections, the moisture contents of which had been previously evaluated using the "Protimeter".

Moisture measurements taken along the entire length of two poles indicate that wick action and rainwater rundown may both have roles to play in the establishment of pole moisture gradients. The distinctive gradient along the total length of the older pole would seem to suggest that it was formed by a rundown of rainwater (although all but the lower measurements were below the fibre saturation point) as water drawn up by wick action would almost certainly be balanced by evaporation before it could reach a height of 25 feet. The importance of rainwater in establishing moisture gradients is further emphasised by the direct relationship shown to exist between annual rainfall figures and pole moisture contents at each of the three geographic locations.

However since a definite moisture gradient did exist in the lower regions of the recently erected pole it was most likely to have been formed by wick action. The relative importance of each mechanism will depend on many various factors including, soil composition, level of water table, structure of pole, climate, height at which moisture content is measured and most certainly the month

of the year in which sampling took place.

The results of this present study show that there is no noticeable difference in nitrogen contents of poles which have been in soils with differing nitrogen contents for up to 50 years. The average figure for pole nitrogen contents at each of the three sites in Britain as well as various sites in Sweden is 0.06%, a figure comparable with nitrogen values of untreated pine blocks used in experiments at this laboratory. The soil nitrogen contents of the three sites vary by as much as 350% and so there would appear to be no relationship between nitrogen levels in poles and soil and little or no nutrient transfer between the two. King, Mowe, Bruce and Smith (1981) have shown that failure of preservative treated wood is always accompanied by nitrogen increases whereas wood which has above threshold levels of preservative shows no increase in nitrogen content. However Uju, Baines and Levy (1981) proposed that nitrogen levels in wood in soil could increase via the transport of soluble nutrients by wick action. Since all the poles in this study, due to effective creosote treatment, are decay free and are all the same wood type there should be no intersite variation in their nitrogen contents unless taken up by wick action from soil. The results in Table 5.2 show that no such nitrogen uptake was found.

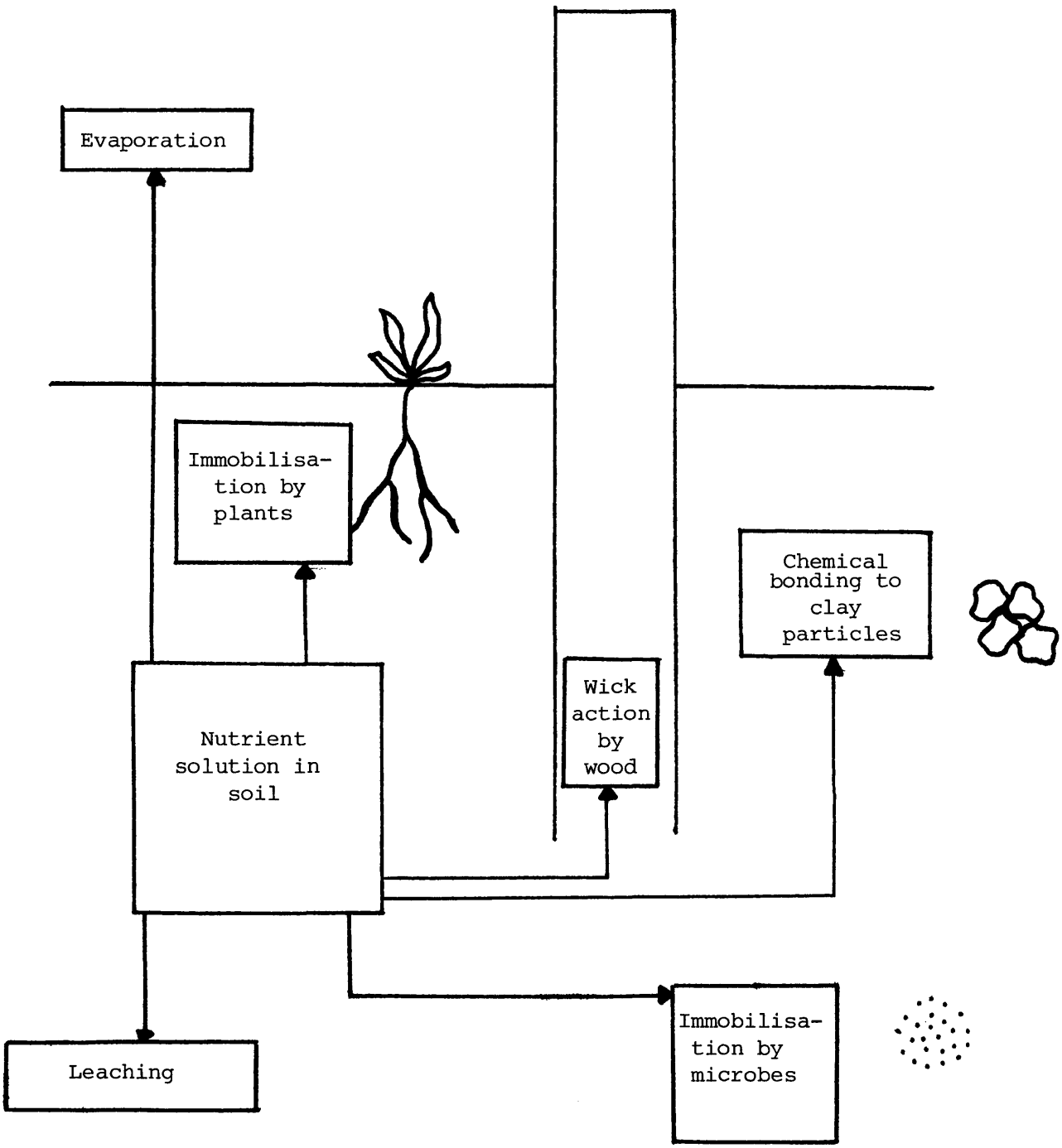
The nitrogen content of soils and in particular the soluble nitrogen fraction is continually changing and is affected by a large number of parameters such as, the type and density of plant cover, the humus content of the soil, the structure and composition of the soil, level of fertilizer application, extent of drainage of the soil as well as the soil microbial constitution. Since such a large number of variables can alter the soil nitrogen level it is most unlikely that any two soils will have identical soluble nitrogen contents.

If wick action is to play a significant role in the uptake of nutrients from soil into wood then it must compete in a passive manner against plants and micro-organisms which are actively utilizing any free nutrients in the soil. Various other passive processes are also continually competing against the wick action of poles to remove any soluble nutrients present in soils. These include leaching and evaporation of free nutrients as well as the chemical bonding of charged nutrients to colloidal clay particles. Figure 5.6 shows a simplified model of the drain on soluble nutrients in soil.

The results of the work undertaken in this chapter conclusively show that

1) Moisture contents of poles in service range from as little as 18% to poles which are totally waterlogged.

Figure 5.6 Diagrammatic representation of the drain on soluble nutrients in soil.



2) No nitrogen uptake occurs from soil into pole interiors at the decay susceptible groundline region.

3) There is no intersite variation in the nitrogen contents of poles.

If biological control of *L. lepideus* using Binab FYT pellets is to be successful then,

1) The biological control fungi *Trichoderma* and *Scytalidium* FY strain must be moisture tolerant over a wide moisture range.

2) A sufficient nutrient supply will need to be inoculated along with the control organisms since no nutrients are transferred from the soil to the groundline region of the poles. Nutrients are therefore limited to those present in green wood which are left in the ray parenchyma cells during the curing of the timber and those added during inoculation of the control organisms. This nutrient limitation will also effect the biomass spread of the commensal fungi in the poles and will determine the quantity of Binab FYT pellets needed to adequately treat the high hazard groundline regions.

It would therefore appear that unless the organisms can produce some form of residual protection within the poles after their death, there will be a limit to the length of time which they can actively survive and continue

to protect the wood. Both *Trichoderma* and *Scytalidium* have been shown to protect wood blocks even after death and this capability would have to be repeated in poles if these fungi are to give protection over long time periods.

CHAPTER 6

VIABILITY AND SURVIVAL OF IMMUNIZING COMMENSALS IN CREOSOTED DISTRIBUTION POLES

6.1 INTRODUCTION

The results of the laboratory studies (Chapters 2 - 4) showed that, both Binab *Trichoderma* and *Scytalidium* FY could overgrow and replace *L. lepideus* on agar and that these organisms could protect pine and lime blocks from attack by that decay fungus even when the control organisms had been killed and the blocks thoroughly leached. Production of both volatile and non-volatile antibiotics have been implicated as the possible mechanisms involved. The results of the laboratory studies also showed that *Scytalidium* FY strain was suppressed by volatiles and antibiotics produced by *Trichoderma* when FYT pellets were plated onto malt agar.

The questions which are raised by the results of laboratory studies are whether in practice the control organisms would survive in creosoted wood under the harsh environmental conditions to which it is exposed. Pure culture studies using biological control organisms are far removed from the natural environment in which interactions with other organisms can play a deciding role in the success or failure of any control system. Klingstrom and Johansson (1973) noted that *Scytalidium album*

Klingstrom and Beyer could inhibit or eliminate *Fomes annosus* in standing spruce and Ricard (1976) has shown that *Trichoderma polysporum* and *Scytalidium* FY could survive in creosoted poles. Dubos and Ricard (1974) have shown that *Trichoderma* species could be established in live peach trees in Spain and Lundborg and Unestam (1980) have successfully established *Trichoderma harzianum* and *Scytalidium album* in Norway spruce trees and have shown survival of these fungi in wood over the severe winter conditions encountered in Scandinavia.

Creosoted wood provides a unique environment with a limited natural flora in which a specific basidiomycete *L. lepideus* is usually responsible for the decay process. As such it provides an ideal target for application of a biological control system.

The objectives of the following experiments were:

1) To determine the capabilities of Binab *Trichoderma* and *Scytalidium* FY strain to grow and become established within on line creosoted transmission poles from an initial inoculation with Binab FYT pellets.

2) To examine the effect if any of pole age, decay status or previous remedial treatment on the percentage isolation (take) of control fungi.

3) To evaluate shelf life of Binab FYT pellets under various storage conditions.

4) To isolate and identify decay causative organisms from poles which had previously been classified as decayed by Electrical board employees.

5) To similarly identify causative organisms of pole top rot.

6) To monitor and classify conditions of storm damaged poles.

7) To relate establishment of control fungi to the moisture contents of poles established in Chapter 5.

6.2 MATERIALS AND METHODS

6.2.1 Growth potential of Binab FYT pellet organisms in the field

One hundred and forty nine creosoted transmission poles, currently in service, situated at three different geographical sites i.e. Glenlethnot (81 poles) and Inchture (35 poles) in Scotland and Ludlow (33 poles) in the West Midlands of England, were inoculated with Binab FYT pellets by Dr. J. Ricard in August 1980 prior to the undertaking of this project.

The object of this experiment was that the inoculated poles should be sampled at intervals to monitor the percentage isolation (hereafter referred to as 'take') of the control fungi with increasing time periods. However reisolation figures from the first sampling (April 1981) of poles at the Glenlethnot and Inchture sites were so low (percentage takes of 20% and 8% respectively) that immediate doubts were raised concerning the viability of the pellets which had been used. The remaining pellets from the batch used to inoculate the poles by Dr. Ricard were tested for viability by dilution plating of the pellets onto 3% malt extract agar. The results of these viability tests showed that the batch of pellets were grossly contaminated with *Aspergillus* spp. and totally unacceptable for application to poles.

As a result of the above findings all the poles at the three locations were reinoculated (as described below) with fresh FYT pellets which had been tested for viability and found to be satisfactory (approximately 10^5 *Trichoderma* particles/gram of pellets). Reinoculation was carried out during August 1981 at the Scottish sites and during June 1981 at Ludlow where a fourth group of 17 poles were also inoculated with the new FYT pellets. Poles at the four sites were then sampled regularly at first three monthly and then six monthly intervals to monitor the establishment of the control organisms.

6.2.1.1 Inoculation procedure for Binab FYT pellets

The following inoculation procedure was used for the inoculation of all poles with FYT pellets during this study:

1) A Mattson (No.4337) augur was drenched with industrial alcohol and flame sterilized using a hand held Ronson blowtorch.

2) The augur was then drilled into the pole at a point 10 cms above groundline at an angle of approximately 30° to the horizontal to ensure that the borer reached the centre of the pole at around groundline level.

3) Using the core extractor the thin wood core was removed and aseptically transferred to a presterilized stoppered tube.

4) The borer was removed, and if required the pole's moisture content recorded using the Protimeter Moisture Meter and specially developed probe.

5) Two 4 mm diameter FYT pellets were inserted into the bore hole and pushed into the centre of the pole using a sterile metal rod.

6) The bore hole was finally plugged using a white plastic plug which was vented to allow an adequate oxygen supply to the pellets.

6.2.1.2 Routine sampling of FYT inoculated poles

The routine procedure for the sampling of FYT inoculated poles was carried out in a similar fashion to the one followed during FYT inoculation with the following exceptions,

1) No pellets were inserted.

2) Poles were sampled at a point 3-4 cms above the point of previous isolation of control fungi taking care to ensure that the augur was inserted at an angle of 30° to the horizontal.

3) Sampling boreholes were plugged with beech dowels.

The regular sampling routine as described above is shown in Plates 6.1a-e.

Plate 6.1a Application of Industrial alcohol to shaft and extractor of Mattson augur.

Plate 6.1b Ignition of alcohol drenched augur to ensure total sterilization

Plate 6.1c Mattson augur in position in a creosoted pole showing angle of insertion of approximately 30° to the horizontal.

Plate 6.1d Extraction of sterile wood core before transfer to glass tube.

Plate 6.1e Beech wood dowel being hammered into pole in order to plug the borehole.

On return to the laboratory the creosoted portions of the cores were removed and the remaining cores were transferred onto plates of 3% malt extract agar and incubated in the dark at 25°C for up to three weeks to allow the growth of any organisms present. (Plate 6.2)

6.2.2 Effect of pole age, decay status and previous remedial treatment on growth of biological control organisms

Fifty five poles spread throughout two sites at Ludlow in the West Midlands were selected because of their particular condition regarding age, decay status and presence or absence of previous 'cobra' treatment. These were split into 5 categories as follows:

1) Poles greater than 20 years old and undecayed (15 poles)

2) Poles greater than 20 years old with decay present (5 poles)

3) Poles greater than 20 years old, 'cobra' treated and undecayed (15 poles)

4) Poles greater than 20 years old 'cobra treated with decay present (5 poles)

5) Poles less than 10 years old and undecayed (15 poles)

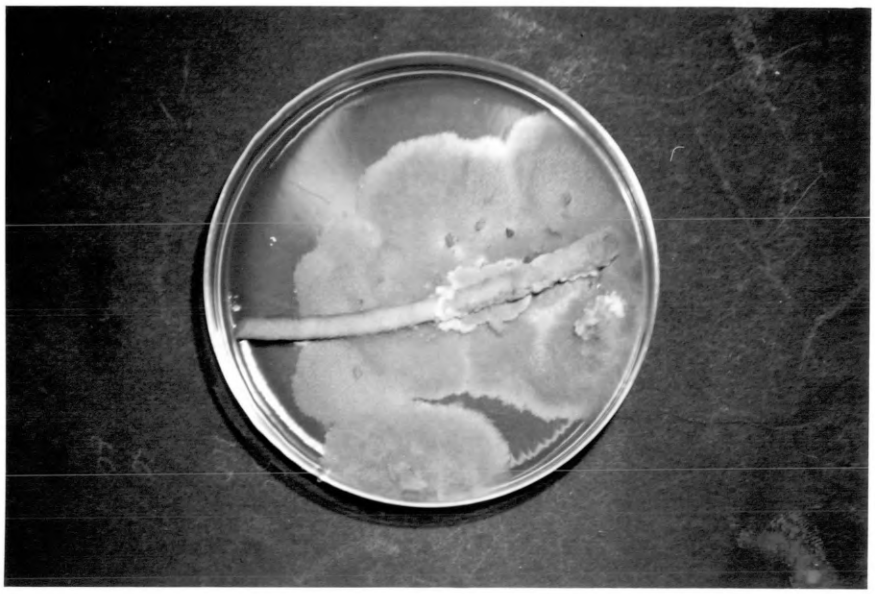


Plate 6.2 Growth of *Trichoderma* from a core removed from a Binab FYT pellet inoculated distribution pole.

These poles were inoculated, as described previously with two 4 mm diameter FYT pellets, in June 1981 and were subsequently sampled at 3 monthly and then 6 monthly intervals until March 1983. Wood core samples were plated onto malt agar as before in order to establish a value for 'take' of biological control fungi.

6.2.3 Shelf life and viability of Binab FYT pellets

Since the Binab FYT pellets were being evaluated as a commercial product for the electrical authorities close liaison was maintained with representatives of the Midlands and North of Scotland Hydro Electric Boards. These collaborators expressed a desire to know what type of storage conditions would be required to ensure no loss of pellet viability. With this in mind the following laboratory quality control tests were carried out.

Bulk samples of Binab FYT pellets were stored under the following three storage conditions:

(a) External storage - On shelf in outdoor garage with varying temperature.

(b) Internal storage - In drawer within college, varying temperature with minimum of 20°C.

(c) Fridge storage - On shelf in fridge with constant temperature of 5°C.

At periods of 0, 1, 2, 3, 6, 12 and 21 months samples were removed from each batch and used to establish the viability of the pellets at each particular time. One gram of pellets was carefully weighed before being transferred to a Waring blender where they were mixed with 100 mls of sterile distilled water. By removing 1 ml aliquots and transferring them to 9 ml distilled water blanks a serial dilution was set up. 0.1 ml samples were removed from the dilution series and pipetted onto the surface of plates of 3% malt extract agar where they were spread to form a lawn over the surface of the medium. Each dilution was plated onto 4 replicate plates and three one gram samples were removed from each storage group at each sampling period, giving a total replication of 12. All plates were incubated in the dark at 25°C for 2 to 3 days at which time those plates giving the most reasonably manageable numbers of *Trichoderma* cultures were counted. Mean figures for each storage group were then calculated.

At some of the later sampling periods viability of some of the stored pellets had fallen so low that dilution factors of even 10^{-2} had become obsolete. In these cases, the one gram samples of pellets were added to 10 mls of distilled water rather than 100 mls and 1 ml aliquots were used for plating out purposes rather than 0.1 mls. Since it was not practical to spread a 1 ml

sample as a lawn onto a malt agar plate the 1 ml samples were placed into empty petri dishes and mixed with molten agar to form pour plates.

In addition to the above mentioned storage conditions a fourth type was also examined. Binab FYT pellets were transferred from the fridge (5°C) to the deep freeze (-18°C) for the final six months of the trial. This was carried out at the suggestion of the electrical authorities as these conditions most closely represented the electrical boards present storage facilities.

A similar procedure was carried out on all pellets which were to be inserted into on line distribution poles as part of the project to maximise the potential success of the control system.

6.2.4 Isolation and Identification of decay causative organisms in poles

Distribution poles situated on line are periodically examined by employees of the electrical authorities to monitor the presence of decay and the need to replace poles. This work is carried out by what is commonly known as the "hammer test" whereby the pole is hit around the groundline region with a hammer and by listening for sound changes the linesman can detect decay. If found, a boring is taken to verify the presence of a decay pocket

before the pole is stamped S - suspect or D - decayed. This technique relies heavily on the ability and experience of the linesman and detects decay only after a cavity has been produced in the pole. The production of a cavity may mean that the decay fungus has been resident in the pole for many years. (see Chapter 7).

In order to isolate and identify the causative organism of decay in on line poles all the poles involved in the current study which were labelled as either S or D were sampled. Cores were removed from within the groundline region of the poles as before using a Mattson augur and were transferred to sterile glass tubes. The cores were then plated out onto 3% malt extract agar incorporating benomyl at 4 ppm. This media allows the growth of basidiomycetes but does not permit the growth of other pole resident fungi. Basidiomycetes isolated using this technique were then identified by both the use of Noble's (1964) key for the identification of 'Cultures of Wood-Inhabiting Hymenomycetes' and by comparison of the cultural and microscopic characteristics of fungal isolates with those of a British strain of *Lentinus lepideus* supplied by the Forest Products Research Laboratory, Princes Risborough.

6.2.5 Isolation and Identification of decay causative organisms in pole tops

Although almost entirely all decay occurs in the high hazard groundline regions of poles some decay has been found in pole tops particularly in the West of Scotland. In order to identify the causative organism and to determine if the same fungus is responsible for both top-rot and ground level rot in poles all the decayed pole tops from the North of Scotland Hydro Electric Board region were sent to this laboratory for examination.

Five pole top sections showing various degrees of decay, although all fairly extensive, were examined for presence of decay fungi. Thin sections of wood were removed from that part of the wood adjacent to the totally decayed rot pocket, wood which itself shows no macroscopic signs of decay. A flamed scalpel and forceps were used to ensure total sterility. Wood was removed from this area since it is here that the fungus is most likely to be active producing incipient decay and is therefore more likely to be isolated successfully. Fewer contaminant organisms are also likely to be present in this area of the wood. Approximately 20-30 sections were removed from various sites on each decayed pole and plated out onto 3% malt extract agar incorporating Benomyl at 4 ppm. Any

basidiomycetes isolated on this selective media were then identified using Nobles (1964) key and by comparison with stock cultures of decay fungi.

6.2.6 Decay status of storm damaged poles

During the winter of 1981-82 many of the more remote areas within the North of Scotland Hydro Electric region underwent some of the most severe weather conditions under record with temperatures being below freezing for weeks on end and snow storms and blizzards being most frequent. During this period one particular distribution line in the North East of Scotland, erected in 1952, was completely flattened during one such storm and 37 poles were broken or damaged. The 37 poles were transported to the electricity board's depot at Arbroath where with the help of a board employee with a power chain saw an examination for decay presence was carried out on each pole. The poles were examined at the point of failure and if decay was detected either visually or by hammer test the pole was sliced to determine the length of the decay pocket.

6.3 RESULTS

6.3.1 Growth potential of Binab FYT pellet organisms in the field

The results of percentage 'take' for *Trichoderma* at

each of the four sites at each sampling period together with an accumulative 'take' value for each site are shown in Table 6.1. These indicate that *Trichoderma* can be readily established in creosoted poles in the field, with up to 94% of poles inoculated with Binab FYT pellets showing establishment of this fungus. *Trichoderma* was never isolated from cores removed prior to inoculation with FYT pellets. *Scytalidium* FY strain was not recovered from any of the poles. The results also indicate that although the figures for 'take' at each sampling period vary to a large extent, the cumulative figure for *Trichoderma* 'take' has increased over the entire period since inoculation and indeed may well increase further. There is little noticeable difference between the values for *Trichoderma* isolation between the four sites however the value for the Ludlow District lines is slightly lower than that recorded for the other three locations. On one occasion *Trichoderma* was seen to sporulate on the outside of the untreated beech dowels used to plug the pole after sampling (Plate 6.3).

6.3.2 Effect of pole age, decay status and previous remedial treatment on growth of biological control organisms

Scytalidium FY strain was not recovered from any of the 55 poles inoculated with Binab FYT pellets during this

Site	Sample Date	% 'Take' of *Trichoderma* at time of sampling	Cumulative % 'take' of *Trichoderma* at any time	Date of Pellet inoculation
1. Glenlethnot (81 poles)	4/81	20	20	8/80 Reinoculated 8/81
	8/81	35	42	
	11/81	62	73	
	4/82	41	77	
	10/82	69	85	
	4/83	83	90	
2. Inchture (35 poles)	4/81	9	9	8/80 Reinoculated 8/81
	8/81	34	43	
	11/81	57	69	
	4/82	43	80	
	10/82	77	91	
	4/83	60	91	
3. Ludlow Training Site (33 poles)	6/81	45	45	8/80 Reinoculated 6/81
	9/81	79	85	
	3/82	67	91	
	9/82	74	94	
	3/83	63	94	
4. Ludlow District lines (17 poles)	9/81	47	47	6/81
	3/82	29	59	
	9/82	29	71	
	3/83	41	83	

Table 6.1 Percentage of poles from which *Trichoderma* was isolated.

Plate 6.3 *Trichoderma* sporulating on the outside of an untreated beech dowel after growth from untreated interior of FYT inoculated pole.

experiment. Table 6.2 shows the percentage of poles from which *Trichoderma* has been isolated and how this figure is effected by pole age, decay status and presence or absence of previous remedial 'cobra' treatment. These results show that decay presence has no effect on the establishment of *Trichoderma* however both aged poles and the presence of previous remedial treatment have a detrimental effect on the percentage 'take' figures for *Trichoderma*. *Trichoderma* was established in 93% of poles less than 10 years old but in only 80% of poles greater than 20 years old. The same fungus was established in 80% of poles which had no previous remedial treatment but in only 53% of similarly aged 'cobra' treated poles, however the control fungus was established in 80% of cobra treated poles which had shown decay. As in the previous experiment the cumulative percentage 'take' of *Trichoderma* increased throughout the time period of this study and may be expected to continue to increase.

6.3.3 Shelf life and viability of FYT pellets

Although the presence of *Scytalidium* FY strain arthrospores were verified by microscopic examination of a solution of FYT pellets no growth of this fungus was ever recorded during dilution plate counting to measure

Treatment Group	Sample Date	% 'Take' of *Trichoderma* at time of sampling	Cumulative % 'take' of *Trichoderma* at any time
1. Poles greater than 20 years old and undecayed (15 poles)	9/81	47	47
	3/82	20	53
	9/82	40	73
	3/83	27	80
2. Poles greater than 20 years old and decayed (5 poles)	9/81	60	60
	3/82	60	80
	9/82	60	80
	3/83	60	80
3. Poles greater than 20 years old undecayed and 'cobra' treated (15 poles)	9/81	20	20
	3/82	13	27
	9/82	20	40
	3/83	40	53
4. Poles greater than 20 years old 'cobra' treated and undecayed (5 poles)	9/81	40	40
	3/82	0	40
	9/82	60	60
	3/83	60	80
5. Poles less than 10 years old and undecayed (15 poles)	9/81	80	80
	3/82	60	87
	9/82	20	93
	3/83	60	93

Table 6.2 Effect of pole age, decay status and previous remedial treatment on *Trichoderma* take in poles inoculated with Binab FYT pellets in June 1981.

pellet viability. Because of this the growth of *Trichoderma* cultures were used as an indicator of viability of FYT pellets.

Table 6.3 shows the effects of storage conditions on the viability of FYT pellets. One gram of Binab FYT pellets as supplied by Bioinnovation Ab Binab was shown to contain approximately 6.0×10^5 viable particles (i.e. spores or mycelial fragments of *Trichoderma*). This level of viability remained high in those pellets stored at 5°C and over the 21 month storage period had fallen only by a factor of 10. However those pellets stored in external and internal stores showed a total loss of viability over the 21 month period with the internally stored pellets showing rapid loss of viability within the first two months of storage. Pellets stored in the deep freeze for a period of six months showed no apparent loss of viability (5.6×10^4 viable particles recorded after storage at -18°C). Each batch of FYT pellets which were used for pole inoculation were shown to have at least 2.0×10^5 viable *Trichoderma* particles per gram.

Date of Sampling	Storage Site		
	Fridge	Internal Storage	External Storage
August 1981	6.0×10^5	6.0×10^5	6.0×10^5
September 1981	4.8×10^5	3.4×10^4	9.0×10^4
October 1981	1.9×10^5	3.0×10^3	1.0×10^5
November 1981	2.6×10^5	4.4×10^3	3.6×10^4
February 1982	2.7×10^5	2.2×10^3	6.9×10^4
August 1982	5.6×10^4	8.4×10^1	1.8×10^3
May 1983	6.0×10^4	2.0×10^0	1.9×10^1

Table 6.3 Effect of storage on viability of FYT pellets (all results presented are viable *Trichoderma* particles/gram of pellets).

6.3.4 Isolation and Identification of decay causative organisms in poles

During this study to isolate and identify decay causing organisms a total of 13 poles classified as decayed by the Electrical Authorities were sampled. Basidiomycetes were isolated from five poles and in each case were identified as *L. lepideus*. In addition during on line sampling of FYT inoculated poles the same fungus was isolated from a further three poles. Creosoted timber provides an environment which contains limited numbers of organisms. Table 6.4 shows the types and frequency of isolation, on 3% Malt Extract Agar, of the various organisms found during sampling of on line poles. Fungal species were identified by cultural and microscopic characteristics using Barnett's "Illustrated Genera of the Imperfect Fungi" (1955).

6.3.5 Isolation and identification of decay organisms in pole tops

Five poles showing extensive top rot were sampled and Basidiomycetes were isolated from three. In each case the fungus was identified as being similar to the *Lentinus lepideus* found to be the major cause of groundline decay. Plate 6.4 shows a pole top with extensive decay on which the white *L. lepideus* mycelium is clearly visible.

Pole resident fungus	Percentage of poles from which fungus was isolated during routine on line sampling
Cladosporium resinae	74.7
Fusarium spp.	16.6
Bacteria spp.	15.7
Phialophora sp.	11.1
Penicillium spp.	6.5
Scytalidium sp.	2.8
Apospharia sp.	2.8
Mucor sp.	2.3
Sclerophoma sp.	2.3
Paecilomyces variotii	2.3
Aspergillus spp.	1.8
Cephalosporium sp.	1.8
Chaetomium sp.	1.4
Wardomyces sp.	1.4
Geotrichum sp.	0.5
Thielaviopsis sp.	0.5
Cunninghamella sp.	0.5

Table 6.4 Frequency of isolation (%) of non decay organisms from creosoted distribution poles.

Plate 6.4 Pole top showing extensive decay caused by *L. lepideus*.

6.3.6 Decay status of storm damaged poles

The results of the study of the 37 poles brought down by storms are shown in Table 6.5. These show that of the 37 poles which failed 22 had detectable decay pockets while 15 were sound. Of the 22 poles showing decay 21 poles failed at groundline indicating that this is the high hazard decay area, however of the 15 undecayed poles 9 failed at heights of greater than 6 feet from groundline.

Of the total failed poles 17 had been previously treated by the 'cobra' technique, of these 47% were found to have decay pockets. In comparison with this, of the 20 remaining non 'cobra' treated poles 70% were found to contain measurable decay pockets. The extent of decay found varied largely between poles, in some cases decay pockets of greater than fifteen feet in length were found.

6.4 DISCUSSION

The results of on line field trials were most encouraging with *Trichoderma* being established in up to 94% of the poles. More significantly perhaps the fungus was still viable three years after inoculation of FYT pellets without the addition of further nutrients to the poles in the intervening period. Therefore provided the

Pole No.	Height of point of failure (ft)	Decay presence	'Cobra' treated	Degree of creosote treatment	Length of decay pocket (ft)	Decay area expressed as percentage of untreated wood
1	Groundline	√	√	***	1.5	3
2	Groundline	√	√	***	3.0	10
3	Groundline	√	√	***	4.0	25
4	Groundline	√	√	***	3.0	30
5	Groundline	√	√	***	4.0	60
6	Groundline	√	√	*	2.0	15
7	Groundline	√	√	*	3.0	30
8	Groundline	√	√	****	2.0	3
9	Groundline	√		**	3.0	60
10	Groundline	√		**	6.0	60
11	Groundline	√		**	25.0	95
12	Groundline	√		**	0.5	3
13	Groundline	√		**	4.0	8
14	Groundline	√		*	1.0	5
15	Groundline	√		*	12.0	35
16	Groundline	√		*	6.0	25
17	Groundline	√		****	2.0	3
18	Groundline	√		***	8.0	20
19	Groundline	√		****	3.0	3
20	Groundline	√		*	3.0	10
21	3.0	√		**	1.0	1
22	5.0	√		*	20.0	35
23	Groundline		√	***		
24	Groundline		√	****		
25	Groundline		√	**		
26	8.0		√	****		
27	10.0		√	*		
28	Top		√	****		
29	Top		√	****		
30	6.0		√	***		
31	6.0		√	**		
32	Groundline			*		
33	Groundline			*		
34	10.0			**		
35	10.0			****		
36	2.0			****		
37	1.0			*		

**** Good creosote penetration. * Poor penetration.

Table 6.5 Report of condition of storm damaged poles.

antagonism between *Trichoderma* and *L. lepideus* shown to take place in the laboratory can be repeated in the interior of creosoted poles Binab FYT pellets may offer a viable alternative to other remedial processes.

The ability of *Trichoderma* spp. to grow in creosoted poles has been previously established (Ricard, 1976; Morris and Dickinson, 1981). The former author achieved figures of between 3 and 90% for reisolation of *Trichoderma* at various sites in Sweden and Denmark. Scots pine poles were inoculated with a nutrient based flour containing propagules of *T. polysporum* and *T. viride* and sampled 3 months later to test for establishment of these fungi. Lack of moisture was blamed for low percentage reisolation figures at some sites however the results of this study show that *Trichoderma* can be established even in poles with moisture contents as low as 18%. The results of this present study show percentage reisolation figures comparable with the highest achieved by Ricard in Sweden however these were only established after a period of 30 months. The continual increase in the percentage 'take' figures shows that factors other than time are governing the rate of establishment of the *Trichoderma*. These may include the following:

1) Temperature

Results recorded at each intermediate sampling period show that higher percentage 'take' figures were

recorded for *Trichoderma* when sampling took place in Autumn rather than early Spring. This result indicates that the *T. polysporum* and *T. harzianum* present in the Binab FYT pellets appear to grow better at that particular time of year. The seasonality of *Trichoderma* spp. in forest soils was shown by Widden and Abitol (1980) to be directly related to the temperatures at each particular time of year. These authors found that *T. polysporum* was more prevalent over the Autumn period, its abundance increased from August to September, remained high until January before it declined till finally in April it was absent. *T. harzianum* however was found to be dominant over the warmer July - October period. Although temperatures within the groundline regions of poles will be higher than those of the soil during Summer and lower during Winter the seasonality of Binab *Trichoderma* spp. in the poles agrees closely with the findings of Widden and Abitol (1980) in forest soils.

2) Moisture

The wide range of moisture contents described in Chapter 5, had little effect on the establishment of *Trichoderma* from the Binab FYT pellet inoculation since growth of this fungus was recorded over the entire moisture range. However on only one occasion did the moisture content of a pole appear to have a prohibitive effect on the establishment of *Trichoderma*. The particular

pole involved was completely saturated with water and no growth of *Trichoderma* was produced, probably due to oxygen limitation in the sodden pole. However apart from this exception the results indicate that the moisture content of poles is not a limiting factor on the establishment of the control fungus *Trichoderma* however it may determine the rate of spread within the pole.

3) Pole status

Even though the presence of 'cobra' salts, left in poles after remedial treatment, do not totally inhibit the establishment of *Trichoderma* they do substantially reduce the rate at which it takes place. This effect cannot be due to effects of the fluoride salts present in the 'cobra' treating solution since Lindgren and Harvey (1952) sprayed log sections with a solution of fluoride salts to successfully encourage growth of *Trichoderma* species. All the poles used in this survey had undergone 'cobra' treatment at least 5 years prior to the inoculation of FYT pellets and the 'cobra' salts had therefore been subject to leaching over this period. Differences in amounts of salts leached from individual poles may in part suggest why *Trichoderma* is more readily established in some poles rather than others. The presence of decay pockets in poles has no effect on the rate of establishment of *Trichoderma*.

4) Resident Organisms

The amount of pole resident organisms may have an effect on the speed at which *Trichoderma* can invade the untreated heartwood and sapwood regions of creosoted poles. The results of this chapter provide some circumstantial evidence that this may in fact be true. It is reasonable to assume that the older the pole the more likely it is to have a larger resident population and therefore should reduce the rate of *Trichoderma* spread providing the rate is influenced in this manner. The percentage 'take' of *Trichoderma* was indeed higher in poles of less than 10 years of age than poles which had been on site for more than 20 years even although both sets of poles were inoculated at the same time.

5) Pellet viability

Although the viability of each batch of pellets was measured prior to inoculation, there was no guarantee that the two pellets inserted into every pole contained a uniform number of viable *Trichoderma* propagules. Thus variability between Binab pellets would almost certainly result in slight differences in the rate of spread of the control fungus.

6) Sampling procedure

Sampling procedure does not have any effect on the true rate of *Trichoderma* spread however it can have on the recorded figure. Since the poles involved in this

study are at present carrying live transmission wires there was obviously a limit to the amount of cores which could be removed. Due to this limitation only one core was removed at each sampling period and depending on the internal spread of *Trichoderma* its detection was not guaranteed.

Results from sampling of on line poles labelled as containing decay showed that the organism responsible was a strain of *Lentinus lepideus*. This agrees with Cartwright and Findlay (1958) who identified this fungus as being the most commonly isolated basidiomycete from creosoted timbers due to its ability to tolerate high levels of that preservative. This fungus was isolated by King and Penn (1975 unpublished data) during sampling of creosoted distribution poles for the Midland Electricity Board. During this study the fungus was also isolated from a further three poles which showed no apparent signs of decay and had not been classified as being decayed during previous electricity board testing. These results suggest that *L. lepideus* is present in substantial amounts before any significant decay pockets are formed and thus seriously questions the use of the hammer test as a method of establishing decay presence.

The establishment in wood of large amounts of basidiomycete mycelium prior to the production of significant weight losses was first postulated by Gillespie and Hulme (1970), who postulated that the basidiomycetes survive by utilizing any soluble nutrients present in the wood. Hulme and Shields (1970) suggested that *Trichoderma* could be used to control decay fungi by competition for such nutrients. The fact that *L. lepideus* appears to become established in poles in this fashion suggests that it may therefore be susceptible to control by *Trichoderma*.

Although pole top rot is a relatively minor occurrence when compared to decay in the high hazard groundline regions of poles it is nevertheless a problem in some regions in the West of Scotland. The results of this study indicate that the same *L. lepideus* strain is responsible for decay in both situations. It may be that due to the higher rainfall in the West of Scotland moisture gradients in poles rise to a greater height than those found in poles in other areas of the North of Scotland Hydro Electric Board region. This could produce poles in which moisture contents were more conducive to growth of *L. lepideus* at positions nearer the pole top. This is however purely speculation and further study is needed to establish the cause of this problem.

Although sixteen different fungal genera and several species of bacteria were isolated during the routine sampling of the on line poles only *Cladosporium resinae* was consistently isolated supporting the view that the interiors of creosoted poles are a unique environment with a limited natural flora. The isolation of *C. resinae*, as by far the most common inhabitant of creosoted poles, is in disagreement with the findings of King and Penn (1975) who did not list that fungus as among the most common isolates from their sampling of creosoted poles in the Midlands region of England. These authors identified a *Paecilomyces* species as the most common isolate, occurring in about 60% of cores taken from poles, however this fungus was isolated from only 2.3% of the poles sampled during this present study. There is some agreement between the findings of the two studies in that *Penicillium*, *Phialophora* and *Fusarium* species are amongst the more common isolates in both investigations.

The fact that *C. resinae* is the most common inhabitant of creosoted poles is not surprising since this fungus has long been associated with wood treated with creosote and coal tar oils as well as with certain petrochemical products. Christensen *et al* (1942) isolated this fungus from creosote treated poles and railway sleepers and these authors tentatively classified the fungus as *Hormodendron resinae*. The terms *Hormodendron resinae* and *Amorphotheca*

resinae are now synonymous with *Cladosporium resinae*. Line (1977) isolated only two fungal isolates from soil which had been recently treated with creosote, these were *Fusarium solani* and *C. resinae*. Since these two genera are the most commonly isolated during this study it would appear as expected that their frequency of isolation from poles is directly related to creosote tolerance by these organisms. *C. resinae* can grow on agar media containing up to 10% creosote (Christensen *et al*, 1942) and more recently has been causing major problems due to its ability to grow and sporulate in supersonic aircraft fuel tanks (Hill and Thomas, 1975).

No *Scytalidium* FY strain was recovered from any poles which were inoculated with Binab FYT pellets and only *Trichoderma* was obtained from the dilution plating of pellets during viability studies. Microscopic examination however verified the presence of *Scytalidium* arthrospores in the FYT pellets. The fact that no growth of this fungus was achieved supports earlier laboratory findings which showed that the faster growing *Trichoderma* fungus could produce volatiles and soluble antibiotics which could suppress the growth of *Scytalidium* FY strain.

The results from the survey on storm damaged poles reinforce the belief that most poles which are decayed ultimately fail at the groundline which due to moisture content of the wood is the position where decay is most

likely to occur. The fact that fifteen of the thirty seven poles which failed had no decay present is a testament to the severity of the conditions. The majority of these undecayed poles failed at points 5-10 feet above groundline and this is directly due to the effects of storm force winds acting on poles which have lost their ability to sway since they are held rigid due to the frozen state of the pole and surrounding soil. This physical breakage of the wood is easily recognized by the characteristic splintered breakage pattern produced. The effectiveness of the 'cobra' process is evident from the results of this survey and since there is no means of knowing whether decay pockets were present before 'cobra' treatment the results presented here may minimise the control achieved by this remedial process.

Binab FYT pellets were shown to be relatively easy to store without any drastic loss of viability and this together with the ease with which they can be applied make them an attractive alternative remedial process for the electrical authorities. At present some electricity boards use the 'cobra' process for the remedial treatment of poles however due to the toxic properties of the salts used, board employees will not treat the poles and hired contractors have to be brought in to carry out this work.

The results of the experiments undertaken in this chapter show that,

1) *Trichoderma* can be established in up to 94% of poles inoculated with Binab FYT pellets.

2) The establishment of *Trichoderma* is reduced in older poles and those which have previously undergone 'cobra' treatment although is unaffected by the decay status of the poles.

3) Binab FYT pellets can be stored at temperatures of -20° and 5°C without only loss of viability however storage at higher temperatures produces a reduction in the numbers of viable propagules per gram of pellets.

4) *L. lepideus* was identified as the primary organism responsible for decay in standing creosote treated poles, both at the groundline regions and in pole tops.

5) *Trichoderma* can be established in poles with moisture contents ranging from 18% to those which are almost totally waterlogged.

6) The majority of poles which contained internal decay caused by *L. lepideus* failed at the groundline proving that this is the high hazard region of the poles.

CHAPTER 7

DISTRIBUTION OF BINAB IMMUNIZING COMMENSALS AND *LENTINUS LEPIDEUS* IN CREOSOTED DISTRIBUTION POLES

7.1 INTRODUCTION

Results from laboratory studies described earlier indicated that *Trichoderma* and *Scytalidium* species had the potential to control *L. lepideus* under laboratory conditions i.e. an environment which is ideally suited to the control fungi. The creosoted pole however is an environment in which control mechanisms actively demonstrated in agar by biological control agents, e.g. antibiotic production, may no longer operate due to nutrient limitation. Baker and Cook (1974), among others, have highlighted the high failure rate of biological control systems in the field even when presumptive laboratory tests had shown them to be successful. Moreover, when commercial restraints are applied even fewer of the control systems are found to be cost effective. The final stage in the research and development of any biological control system is therefore the monitoring of interactions between the competing organisms in the field environment.

Fieldwork described in Chapter 6 showed that *Trichoderma* was established in a large proportion of the poles inoculated with Binab FYT pellets, however the sampling technique employed gave little indication of the spread

of this organism within the poles. Since the inoculation process as well as the effectiveness of the control agents was under study it was essential that the spread of *Trichoderma* from the initial single inoculation point be quantified. Furthermore the experiments undertaken in this chapter were also carried out to show the spread of *Lentinus lepideus* in creosoted poles and how colonisation of the wood by this fungus was influenced by inoculation with Binab FYT pellets.

7.2 MATERIALS AND METHODS

7.2.1 Pole Treatment Groups

Sixty transmission poles on site at the Midlands Electricity Board's Linesman Training School at Ludlow were selected for use in this experiment since these poles had a short lifespan (2-3 years) and would be available for destructive sampling when required. This short lifespan is due entirely to excessive surface wear caused by the spiked climbing boots worn by the trainee linesmen and not by microbial decomposition. These poles were divided into four groups of fifteen for different biological treatments. Due to availability of poles, the fact that they were in service and the time constraints of the project, ten poles from each of the above treatment groups were sectioned extensively to monitor the extent of fungal colonisation. The remaining

five poles per treatment group were sampled by the removal of a single wood core at six monthly intervals for the duration of the project. Due to the working requirements of the training site some poles were removed and replaced before decay and/or control fungi had been allowed sufficient time to permeate through the wood. When this occurred the relevant poles were replanted as stubs in a nearby grass covered field (Plate 7.1). Such stubs were buried to the same depths to which the original poles had been embedded to ensure that the inoculation point remained at ground level. In general a minimum incubation period of 12 months was observed before any of the poles were extensively sectioned.

(i) Extent of colonisation of poles by Binab FYT organisms (Group 1)

These poles were inoculated with two 4 mm diameter Binab FYT pellets in June 1981 and were sampled in December 1981 to monitor the establishment of the control fungi. Inoculation and sampling procedures were identical to those described previously. Neither *Trichoderma* nor *Scytalidium* FY strain were isolated from the wood cores removed from the poles prior to pellet inoculation. When these poles were routinely replaced, providing a minimum incubation period of 12 months had elapsed, sections approximately 60 cms in length were

Plate 7.1 Pole stubs at the Midlands Electricity Board training site at Ludlow.

Plate 7.2 Agar plate with six samples removed from a creosoted stub. Each sample has its own code which represents its position in the pole.

removed from the groundline regions and transported to the laboratory for extensive sampling. These sections were extracted by sawing through the pole at positions 25 cms above and 35 cms below the inoculation point. In addition, one pole which had been inoculated with Binab FYT pellets during on line studies in Scotland but later removed as part of routine transmission line maintenance was also transported to Dundee for sampling. A total of eleven stubs were extensively sampled in this treatment group.

(ii) Extent of colonisation of poles by *Lentinus lepideus* (Group 2)

Beech dowels colonised with *L. lepideus* were prepared by placing autoclave sterilized dowels onto actively growing cultures of the decay fungus on malt extract plates and incubating for periods of 6-8 weeks. Randomly selected dowels were sectioned and microscopically examined to verify that the fungus had penetrated into the wood. Fifteen poles were inoculated with such *L. lepideus* infested dowels in June 1981. The inoculation process was similar to that used for Binab FYT pellet inoculation. A wooden core was removed from the groundline region of each pole using a Mattson augur and the *L. lepideus* infested dowel was inserted into the centre of the pole.

After insertion of the basidiomycete the borehole was plugged using a vented plastic plug. No *L. lepideus* was recovered from wood cores removed from the poles prior to inoculation of the decay fungus.

Preliminary studies, undertaken in September and October, 1981, to monitor the establishment of *L. lepideus* in such poles however indicated that the fungus was viable in only seven of the fifteen poles originally inoculated. This may have been due to failure to ensure that dowels were inserted into the centre of the poles with the result that creosote leached into the dowels.

In December 1981 those poles from which no viable mycelium was isolated were reinoculated at three points equidistant around the circumference of the pole. Inocula consisted of both beech and pine dowels infected as described above as well as pine sapwood sawdust which had been inoculated with *L. lepideus* and incubated for 6-8 weeks. Care was taken to ensure that the inocula were inserted into the centre of the poles well away from the creosote treated regions. Reinoculation of these eight poles was shown, by further preliminary samplings (March 1982), to be successful and thereafter all fifteen poles were sampled at six monthly intervals for the duration of the study. When the poles were replaced (providing a suitable incubation period had elapsed), as for group 1, the groundline sections were removed and

sent to Dundee for sampling. A total of ten poles in this group underwent extensive sampling.

(iii) Effect of immunizing commensal inoculation (Binab pellet) on subsequent colonisation of poles by *L. lepideus* (Group 3)

Prior to inoculation with Binab FYT pellets cores were removed from the poles to determine whether *Trichoderma*, *Scytalidium* or *L. lepideus* were naturally present, however none of these fungi were isolated. As in group 1, poles were inoculated with Binab FYT pellets in June 1981 and sampled six months later to establish the presence of the immunizing commensals. Once the presence of *Trichoderma* had been established in the poles they were then inoculated with *L. lepideus* in December 1981. As in group (ii) *L. lepideus* inocula in the form of either pine or beech infested dowels or sawdust was inoculated at three points at the groundline region of the poles. Poles were again sampled at six monthly intervals and as they became available ten poles were extensively sampled at the laboratory.

(iv) Effect of subsequent inoculation with Binab FYT pellets on the colonisation of poles by *L. lepideus* (Group 4)

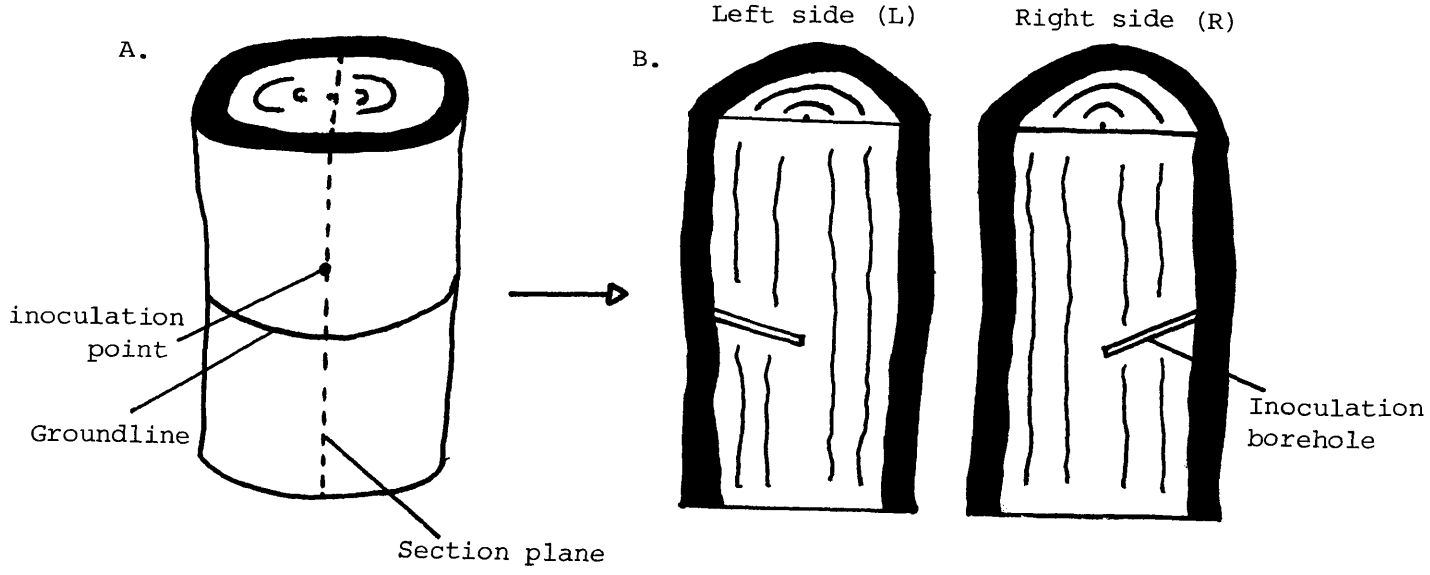
Having established that no *Trichoderma*, *Scytalidium* or *L. lepideus* was present in the poles prior to treatment,

fifteen poles were inoculated as described previously with *L. lepideus* infested beech dowels. This was undertaken in June 1981, however as with group (ii) preliminary samplings in September and October of that year showed that the decay fungus was no longer viable in ten of the poles. As with the other treatment group these poles were reinoculated at three positions with *L. lepideus* infested pine and beech dowels and sawdust. This reinoculation was carried out in December 1981 and further preliminary samplings verified the establishment of the decay fungus. The poles were then inoculated with Binab FYT pellets in May 1982. The Binab pellets were inserted into the poles close to the site of *L. lepideus* inoculation. The poles were then sampled at regular intervals until in June 1983 the groundline sections of ten poles were removed and forwarded to the laboratory for sampling.

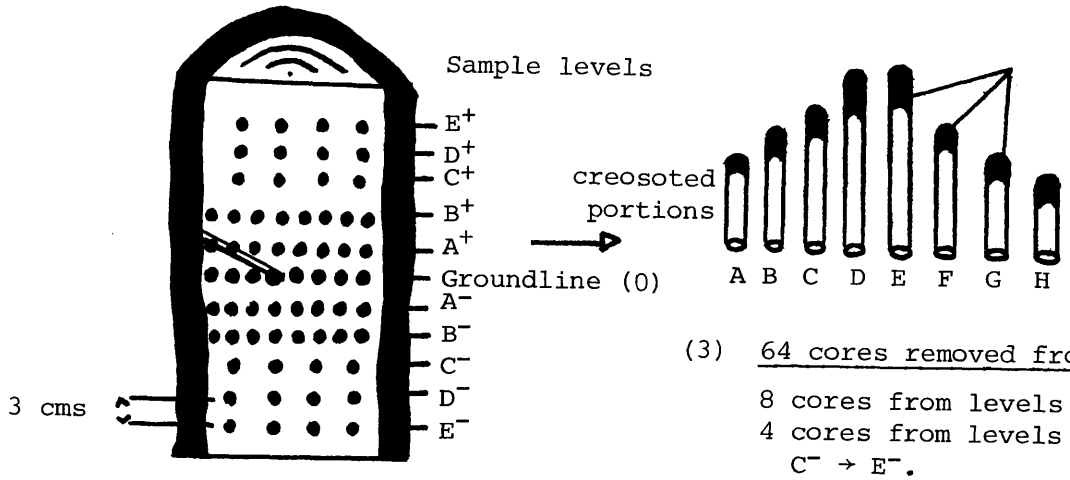
7.2.2 Sectioning of pole stubs and isolation of inhabitants

On arrival at the laboratory in Dundee the location number of each pole was recorded and the stub sectioned as soon as possible. The method of sampling of the pole sections is described below and illustrated in Figure 7.1.

1. Stubs split through inoculation point



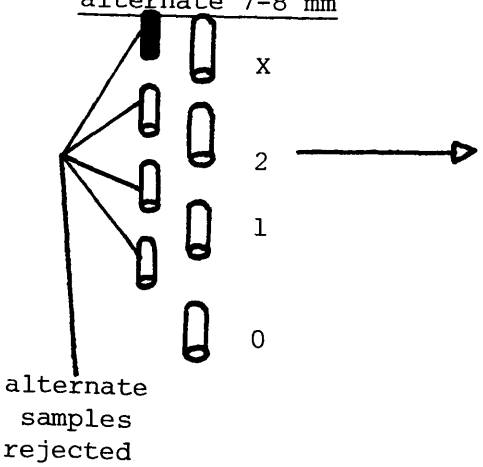
2. Each side extensively sampled using Mattson augur

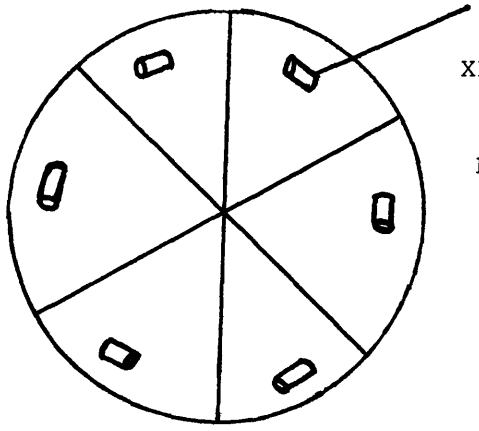


(3) 64 cores removed from each side of pole

8 cores from levels B^- → B^+
4 cores from levels C^+ → E^+ and C^- → E^-.

4. Each core then sampled every alternate 7-8 mm





Example
(Pole XB2, Sample LB⁻D4)
XB2 - Pole location number
L - left side
B^- - sample level B^-
D - core position
4 - sample position

Labelled samples plated onto malt extract agar

Figure 7.1 Procedure used for sectioning of pole stubs.

Using a hammer and masonary chisels each stub was split in half longitudinally where possible with the dissection plane passing through the Binab FYT pellet borehole. In poles inoculated only with the decay fungus the dissection plane was directed through one of the three *L. lepideus* inoculation points (Figure 7.1(1)). Taking each half in turn the groundline was marked in pencil on the newly exposed face of the pole and labelled 'level 0'. Ten other levels each 3 cms apart at increasing heights above and below the groundline were then marked out on the poles. Levels $A^+ \rightarrow E^+$ indicate above groundline locations while levels $A^- \rightarrow E^-$ represent positions below the groundline (Figure 7.1(2)).

Using a Mattson augur equally spaced cores (approximately 2 cm centres) were removed across the radial face at each level. Eight cores (A → H) were removed from levels B^-, A^-, 0, A^+ and B^+ while four cores (A → D) were removed from each of the six remaining levels (Figure 7.1(3)). In every case core A was removed from the side of inoculation. Due to the semicircular nature of each half stub the lengths of the cores naturally varied with cores A and H being the shortest while cores D and E were the longest.

The uncreosoted portion of each core was then cut into specimens 7-8 mm long. Starting from the uncreosoted

end of the core alternate samples labelled 0, 1, 2,...X were removed and plated onto malt extract agar. Each petri dish contained six specimens each labelled with its own unique coding (Figure 7.1(4)).

Plate 7.2 shows an agar plate with six coded specimens while Plate 7.3 shows the skeleton remains of a creosoted stub section after it had been fully sampled.

Samples removed from those poles inoculated only with Binab FYT pellets were plated out onto 3% malt extract agar, incubated in the dark at 25°C, and observed daily for growth of both control fungi and resident pole organisms. As soon as immunizing commensals had been positively isolated from any wood samples the relevent sections of agar were quickly removed to prevent cross contamination of other samples in the same petri dishes. The same procedure was required when other spore producing residents such as *Penicillium*, *Aspergillus* and *Mucor* were isolated from wood samples. The remaining samples were incubated for three weeks to allow for the growth of all pole resident fungi. Using the coding system the position of each organism isolated was then mapped onto a diagram representing each of the levels within the pole stub. Each organism was colour coded so that fungal spread could be easily identified. In the rare instances in which two or more organisms were isolated

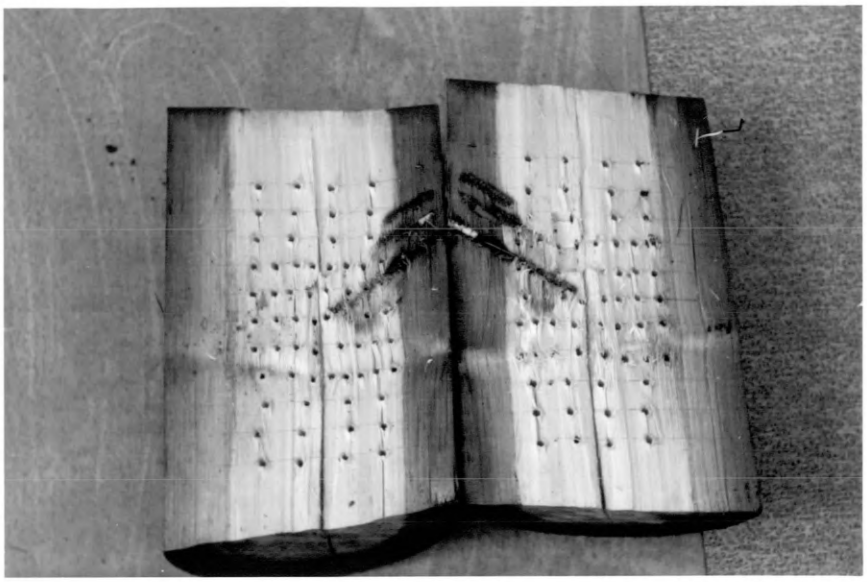


Plate 7.3 Skeleton remains of a creosoted pole section after extensive sampling.

from any single wood sample only the fungus occupying the largest portion of the sample was recorded. However if immunizing commensals were isolated their presence was recorded even though other fungi may have occupied a larger portion of the sample.

Samples removed from poles inoculated with *L. lepideus* were plated onto 3% malt extract agar incorporating benomyl at 4 ppm concentration. This medium allowed the growth of basidiomycetes but did not permit the growth of other resident pole fungi on the agar. These other fungi including *Trichoderma* did however grow and sporulate on the wood blocks and could therefore be identified and their distribution mapped. Distribution of both *L. lepideus* and immunizing commensals could thus be evaluated from samples removed from poles inoculated with both Binab FYT pellets and the decay fungus. All plates were incubated at 25°C for up to four weeks to permit the growth of all the resident organisms. As above colour coded distribution maps were drawn up to represent the distribution of the various organisms present at each level in the pole. Whenever two or more fungi were isolated from the same wood sample only the major occupier was recorded unless *L. lepideus* was present in which case the presence of that fungus was recorded.

Using a computer programme developed by the Department of Computer Studies at Dundee College of Technology a graphic representation of the spread of both control and decay fungi, as well as that of the pole resident organisms, was achieved for all sample levels (Bruce, 1983).

7.3 RESULTS

A total of forty one pole stubs were extensively sectioned and more than 20,000 samples plated out onto malt extract agar. Although *Trichoderma*, *L. lepideus* and many types of pole resident organisms were isolated at no point was *Scytalidium* recovered.

(i) Extent of colonisation of poles by Binab FYT organisms

All eleven poles sectioned were found to contain *Trichoderma*. This included one pole from which no *Trichoderma* had been isolated during the previous routine six monthly sampling visits in which only one core was removed, the control fungus was isolated from only 18 of the total of 583 samples removed from this stub. In almost all of the poles, although *Trichoderma* had reached the upper and lower sampling levels, maximum colonisation was found in and around the groundline regions.

A wide range of other organisms were found to be resident in the poles the most common being *C. resinae*.

These organisms were present in large areas of some of the pole stubs and appeared to inhibit the spread of *Trichoderma*.

The bulk of the results are given in Appendix I(1-11) which represent the colonisation pattern of *Trichoderma* in each of the poles and show how the presence of resident organisms influenced the spread of the control fungus. These also show that spread of *Trichoderma* is contiguous both in any one level and between levels even although these areas were sampled at different times. This continuity was also apparent with pockets of pole resident organisms and indicates that the sampling technique was efficient with little evidence of cross contamination.

Percentage isolation figures for *Trichoderma* and resident organisms were calculated at each sampling level and these are summarised in Table 7.1. Percentage isolation was calculated as follows

$$\text{Percentage isolation} = \frac{\text{Total number of positive isolates}}{\text{Total samples removed}} \times \frac{100}{1}$$

These reinforce the observations made from examination of the distribution maps in that range and spread of *Trichoderma* are shown to vary widely. Percentage isolation of *Trichoderma* ranged from 3.1% to 79%. Spread of the fungus above the groundline inoculation point (43.3%), calculated from the total isolates removed from the above groundline regions of all eleven sectioned poles,

(a)

Sampling Levels	Pole numbers										
	XB5	SCOT	XS	XB3	XB6F	XC3L	D5F	XD4	XC3R	XC4F	XC4B
E^+	0	21.4	18.9	3.0	17.0	26.5	13.6	0	0	45.2	12.9
D^+	0	26.8	18.4	3.3	21.7	27.5	24.4	0	0	22.5	60.9
C^+	5.7	26.8	30.6	0	20.8	40.5	42.5	6.5	7.3	28.2	73.1
B^+	18.6	72.4	11.3	0	31.5	48.2	37.5	10.3	1.3	28.6	78.3
A^+	18.2	81.7	36.5	3.3	29.6	61.4	31.7	6.7	1.4	31.3	88.7
Groundline inoculation point 0	19.5	58.7	38.5	8.2	20.0	36.3	35.0	15.8	8.2	34.1	82.5
A^-	22.7	70.0	20.9	10.2	24.5	42.5	17.9	2.7	5.6	32.9	85.1
B^-	26.3	17.3	11.7	11.6	22.5	29.9	16.1	0	4.1	39.3	90.5
C^-	21.0	3.3	11.9	8.8	23.4	40.5	14.3	0	0	44.4	85.7
D^-	8.3	0	2.6	9.1	29.4	31.6	24.4	0	0	47.6	90.3
E^-	25.7	3.2	2.9	0	20.8	39.0	17.1	2.8	0	47.5	83.3
Total % isolation for all levels	17.0	40.7	20.3	5.7	24.2	40.5	25.6	4.9	3.1	35.4	79.0
Total number of samples	599	531	592	512	785	637	656	593	583	655	491

(b)

Sampling Levels	Pole numbers										
	XB5	SCOT	XS	XB3	XB6F	XC3L	D5F	XD4	XC3R	XC4F	XC4B
E^+	100	66.7	18.9	81.8	76.6	38.2	81.8	100	100	31.7	35.5
D^+	100	63.4	26.3	83.3	65.2	60.0	65.9	89.2	91.7	27.5	26.1
C^+	94.3	63.4	33.3	87.1	60.4	37.8	55.0	90.3	92.7	20.5	15.4
B^+	81.4	25.9	39.4	98.5	61.8	42.2	61.3	77.9	97.4	36.9	8.3
A^+	79.2	18.3	37.8	96.7	61.2	26.5	65.9	72.0	98.6	38.6	1.6
Groundline inoculation point 0	77.9	41.3	24.4	91.8	71.0	38.7	63.8	80.3	89.0	45.1	6.3
A^-	72.7	21.7	25.4	79.7	61.8	33.3	73.8	93.3	94.4	43.5	1.5
B^-	67.5	76.0	22.1	81.2	68.6	45.5	67.8	93.1	95.9	36.9	0
C^-	71.1	93.3	9.5	88.2	70.2	37.8	76.2	97.5	100	35.6	0
D^-	88.9	92.9	18.4	84.8	56.9	44.7	65.9	100	100	19.0	0
E^-	74.3	90.6	23.5	96.9	62.3	22.0	68.6	97.2	100	20.0	0
Total % isolation for all levels	80.5	53.7	26.5	88.7	67.6	38.1	67.5	87.4	95.9	35.4	6.5

Table 7.1 (a) Percentage isolation of *Trichoderma* from creosoted poles inoculated with Binab FYT pellets on 16/6/81 and (b) Percentage isolation of other organisms resident in the same poles.

was almost identical to that recorded below the groundline (41.8%).

Since *Trichoderma* appeared to be isolated more frequently when less pole resident fungi were present the mean percentage isolation of both *Trichoderma* and resident fungi at sampling levels A^+, 0 and A^- (i.e. the immediate vicinity of the inoculation point) in each pole were plotted against one another. Figure 7.2 shows that an inverse relationship exists between the spread of *Trichoderma* and the occurrence of pole resident organisms. Whenever pole resident organisms are present in large numbers *Trichoderma* is not frequently isolated and conversely when numbers of pole resident organisms are low the percentage isolation of *Trichoderma* is high.

One of the eleven poles was found to contain a naturally occurring *L. lepideus* infection. The decay fungus was located at the boundary of the *Trichoderma* pocket (see pole XB5 Appendix I(1)). Microscopic examination of wood sections removed from this boundary region were found to contain large amounts of lysed mycelium.

The five poles in this group which were not sectioned all gave positive *Trichoderma* isolation during routine six monthly sampling. No *Scytalidium* FY strain was isolated from any of the poles.

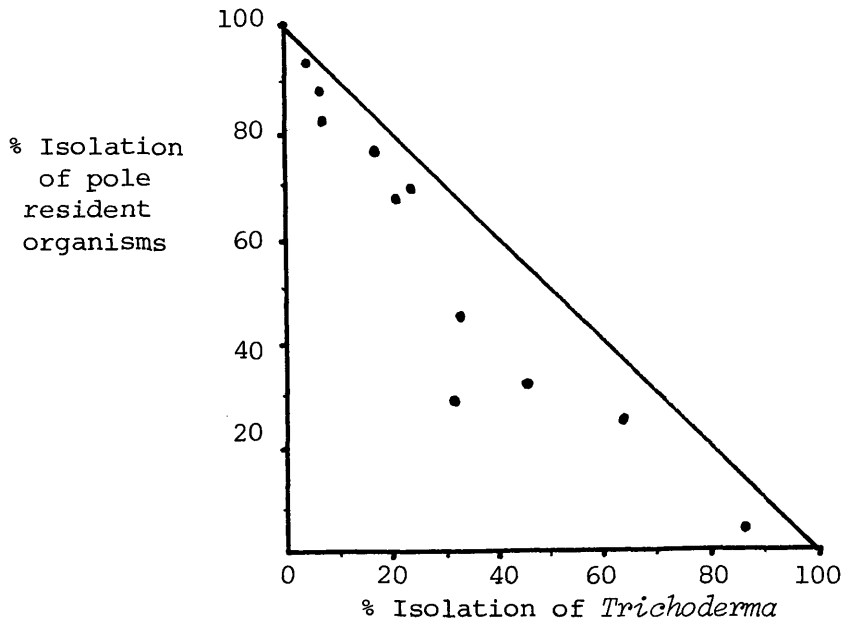


Figure 7.2 The interrelationship between the percentage isolation of *Trichoderma* and the quantity of resident pole organisms in poles inoculated with Binab FYT pellets.

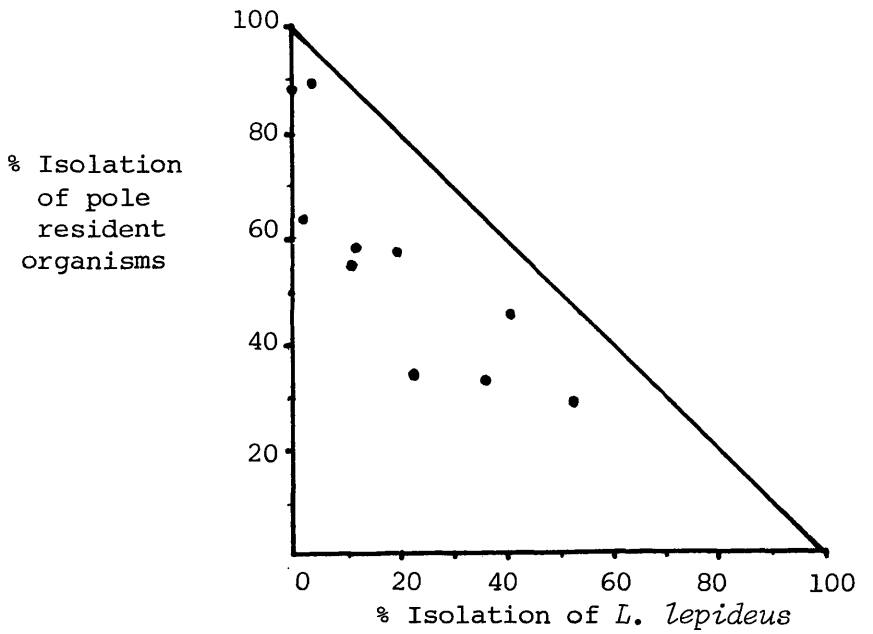


Figure 7.3 The interrelationship between the percentage isolation of *L. lepideus* and the percentage isolation of organisms resident in poles inoculated with *L. lepideus*.

(ii) Extent of colonisation of poles by *L. lepideus*

Of the ten poles sectioned nine were found to contain *L. lepideus*. Appendix II(1-10) shows distribution maps which graphically represent the spread of *L. lepideus* and pole resident fungi within the ten sectioned poles initially inoculated with *L. lepideus*. It is obvious from these diagrams that as in group 1, large quantities of other organisms are resident in the poles. The spread of *L. lepideus* does not however appear to be limited by their presence. In almost all the poles the *L. lepideus* spread had reached the lower sampling levels, however the quantity of the fungus at these points was low compared with the inoculation level. In seven of the nine poles the spread of the *L. lepideus* had not reached a level 15 cms above groundline even although up to 40% of the inoculation level had been colonised. The distribution patterns produced were much more regular than those of *Trichoderma* with expansion in the horizontal plane being almost symmetrical.

Percentage isolation figures were calculated for *L. lepideus* and resident organisms at each sampling level within the poles and are summarised in Table 7.2. These show that the percentage isolation of *L. lepideus* ranged from 3.3% to 46%. As indicated by the maps showing the distribution of the organisms the

(a)

Sampling Levels	Pole numbers									
	XB4	XB1B	XB1F	C4F	B1B	XG2	C4B	H2	XB2	XG3
E^+	0	8.3	0	0	8.7	0	0	2.5	0	0
D^+	0	8.3	0	0	4.3	20.7	0	0	0	0
C^+	0	14.7	5.6	0	10.9	20.0	13.9	7.5	0	0
B^+	0	13.7	18.1	16.2	10.2	27.9	23.5	33.3	1.6	3.6
A^+	0	12.8	29.4	20.5	11.1	30.6	38.6	40.8	1.6	5.1
Groundline inoculation level 0	0	12.0	21.9	33.3	11.1	40.0	42.6	56.0	1.6	3.3
A^-	0	12.3	18.1	10.5	11.2	36.1	43.9	61.3	1.6	4.7
B^-	0	13.9	14.3	10.0	11.1	37.3	42.3	68.3	1.4	5.0
C^-	0	11.1	8.6	6.9	14.6	42.4	44.1	73.2	9.7	8.6
D^-	0	11.1	14.3	3.8	5.0	36.7	48.6	75.0	18.8	0
E^-	0	8.8	15.6	3.4	13.0	40.7	33.3	61.1	9.4	9.7
Total % isolation for all levels	0	12.1	15.3	10.9	10.6	31.8	32.4	46.0	3.3	3.9
Total number of samples	572	580	555	348	697	494	552	630	512	482

(b)

Sampling Levels	Pole numbers									
	XB4	XB1B	XB1F	C4F	B1B	XG2	C4B	H2	XB2	XG3
E^+	77.8	44.4	15.8	13.0	52.2	34.5	85.7	37.5	34.4	86.2
D^+	86.1	33.3	21.2	23.1	72.3	37.9	88.9	18.9	33.3	93.1
C^+	73.0	47.1	13.9	24.0	65.2	46.7	69.4	30.0	48.5	96.6
B^+	60.0	41.1	31.9	37.8	62.5	49.2	67.6	34.5	60.3	92.9
A^+	85.7	60.3	47.1	59.0	50.0	40.3	45.7	38.2	52.4	88.1
Groundline inoculation level 0	91.4	61.3	35.9	55.6	66.7	30.8	47.1	25.3	61.9	95.0
A^-	90.9	42.5	23.6	57.9	64.0	27.9	47.0	20.0	73.4	81.3
B^-	78.7	30.6	30.0	60.0	58.9	34.3	50.7	11.0	73.9	83.3
C^-	52.6	25.0	31.4	31.0	43.9	24.2	41.2	12.2	54.8	71.4
D^-	94.4	27.8	37.1	15.4	65.0	33.3	45.7	13.9	68.8	76.7
E^-	90.9	29.4	34.4	51.7	65.2	25.9	45.5	25.0	84.4	83.8
Total % isolation for all levels	80.1	42.8	30.5	41.9	59.7	35.4	56.0	24.8	60.7	86.5

Table 7.2 (a) Percentage isolation of *L. lepideus* and (b) percentage isolation of resident organisms from creosoted poles inoculated artificially with *L. lepideus*.

average isolation of *L. lepideus* above the groundline inoculation point (29.8%) was substantially lower than that recorded below the groundline (54.6%).

The mean percentage isolations of both *L. lepideus* and resident organisms were calculated at sampling levels A^+, 0 and A^- in each pole and were plotted against one another to determine whether any relationship existed between the spread of *L. lepideus* and the occurrence of pole resident organisms. Figure 7.3 shows that colonisation by *L. lepideus* appears to be effected by the resident organisms. The spread of the decay fungus however is inhibited by resident organisms to a lesser extent than was the spread of *Trichoderma* since in most of the poles in group 2 some groundline regions were found to contain no organisms unlike group 1 in which the percentage isolations of *Trichoderma* and resident organisms accounted for almost all the samples removed from these regions.

Although nine of the ten poles contained significant pockets of the decay fungus, in almost all cases the internal surfaces of the split stub showed no visible signs of decay the wood being apparently sound.

Of the five poles in this category which were not sectioned only one has given a positive isolation of

L. lepideus during routine sampling undertaken in September 1982 and March 1983.

(iii) Effect of immunizing commensal inoculation (Binab pellet) on subsequent colonisation of poles by *L. lepideus*

Although *Trichoderma* had been isolated from all ten poles when sampled prior to *L. lepideus* inoculation, on sectioning only nine were found to contain a viable *Trichoderma* infection. Of the ten poles which were extensively sampled nine contained *Trichoderma* and five contained *L. lepideus*. Five poles contained *Trichoderma* but no *L. lepideus*, four poles contained both the control and decay fungi and 1 pole contained *L. lepideus* but no *Trichoderma*.

The distribution of *Trichoderma*, *L. lepideus* and organisms resident in the poles are presented in Appendix III (1-10). These show that in those poles in which both organisms are present, *Trichoderma* and *L. lepideus* were isolated from spacially separate areas of the pole. The two fungi were never recovered from the same wood specimens even when such samples were removed from the boundary regions between areas colonised by *Trichoderma* and *L. lepideus*.

As with all previously sectioned poles large quantities of other resident organisms were isolated and as in group 1

these resident organisms appeared to limit the spread of *Trichoderma*.

At the time of sectioning *Trichoderma* had permeated throughout the sample area with maximum spread occurring at the groundline. The spread of *L. lepideus* was greater below groundline than above, however maximum isolations were obtained in close proximity to the groundline inoculation levels. As in the previous groups the growth patterns of *Trichoderma* appeared to be irregular while those of *L. lepideus* were more ordered. Percentage isolation figures for *Trichoderma*, *L. lepideus* and resident organisms were calculated as before and are summarised in Table 7.3. These show that the percentage isolation of *Trichoderma* varied widely (10.5% - 49.5%) in the nine poles from which it was recovered. The percentage isolation of *L. lepideus* ranged from 2.6% - 16.2% in the four stubs in which both it and *Trichoderma* were found but was present in 26.7% of the samples removed from that pole from which no *Trichoderma* was recovered. This result would seem to show an inter-relationship between the control and decay fungi however none was apparent from a comparison of their mean percentage isolation figures at the groundline inoculation regions (sample levels A^+, 0 and A^-).

A second basidiomycete was isolated from five of the ten sectioned poles. These included four of the poles from which no *L. lepideus* had been isolated. This fungus

(a)

Sampling Levels	Pole numbers									
	I3	J4	K2L	J3	K2R	J2	I1	I2	I5	J1
E^+	0	8.1	0	17.5	2.5	3.3	0	15.6	12.1	0
D^+	0	5.1	0	2.3	7.0	3.1	18.8	24.1	21.9	11.8
C^+	0	17.5	0	9.3	2.4	12.5	21.9	41.9	22.6	12.1
B^+	0	31.6	5.9	22.8	8.6	19.3	43.8	32.7	25.3	24.6
A^+	0	42.7	15.3	49.3	19.5	18.6	43.3	59.0	33.3	36.1
Groundline inoculation level 0	0	39.0	8.5	43.0	37.3	10.2	48.4	36.1	23.9	39.7
A^-	0	40.3	33.7	39.0	30.1	21.5	52.0	31.3	28.8	44.4
B^-	0	28.4	16.7	20.5	47.6	18.3	62.5	35.5	23.4	54.2
C^-	0	40.0	4.7	10.0	43.9	31.0	68.6	72.4	5.4	56.8
D^-	0	44.4	2.2	7.7	15.4	13.8	83.3	42.9	15.6	42.1
E^-	0	45.4	2.1	7.7	17.1	8.7	84.8	44.0	23.3	54.3
Total % isolation for all levels	0	32.6	10.5	24.9	23.6	15.6	49.5	39.2	23.1	36.3
Total number of samples	565	611	694	642	658	475	546	477	553	554

(b)

E^+	0	0	0	5.0	5.0	0	0	0	0	0
D^+	5.4	0	0	14.0	9.3	0	0	0	0	0
C^+	22.9	0	0	18.6	9.8	0	0	0	0	0
B^+	24.6	3.9	3.5	15.2	17.3	0	0	0	0	0
A^+	55.9	4.0	3.5	5.3	14.6	0	0	0	0	0
Groundline inoculation level 0	37.3	3.9	0	13.9	19.3	0	0	0	0	0
A^-	24.3	3.9	0	9.8	12.0	0	0	0	0	0
B^-	23.6	7.4	6.4	26.5	15.5	0	0	0	0	0
C^-	24.2	7.5	4.7	22.5	14.6	0	0	0	0	0
D^-	23.5	5.6	2.2	28.2	15.4	0	0	0	0	0
E^-	20.6	0	8.3	28.2	17.1	0	0	0	0	0
Total % isolation for all levels	26.7	3.8	2.6	16.2	14.3	0	0	0	0	0

(c)

E^+	38.0	40.5	82.6	47.5	52.5	53.3	36.1	43.8	48.5	34.3
D^+	37.8	53.8	76.9	44.2	25.6	81.3	36.1	69.0	37.5	41.2
C^+	40.0	37.5	59.5	48.8	34.1	68.8	53.1	48.4	54.8	69.7
B^+	49.3	52.6	69.4	45.6	45.7	59.6	48.4	65.5	63.4	59.4
A^+	26.5	45.3	54.1	33.3	40.2	67.8	47.8	41.0	57.6	57.4
Groundline inoculation level 0	37.3	36.4	72.3	34.2	22.9	84.7	48.4	63.9	62.0	55.9
A^-	28.4	37.7	57.3	40.2	15.7	67.7	40.0	54.7	56.2	45.8
B^-	16.7	35.8	67.9	33.7	16.7	70.0	34.7	30.6	50.6	29.2
C^-	27.3	30.0	74.4	50.0	14.6	62.1	28.6	17.2	70.3	24.3
D^-	17.6	22.2	82.2	48.7	17.9	72.4	11.1	17.9	68.8	34.2
E^-	20.6	27.3	72.9	61.5	12.2	69.6	12.1	24.0	56.7	22.9
Total % isolation for all levels	31.2	39.3	70.9	42.2	27.4	69.3	38.5	45.9	57.3	44.6

Table 7.3 Percentage isolation of (a) *Trichoderma* (b) *L. lepideus* and (c) resident organisms from creosoted poles inoculated with Binab FYT pellets followed by the inoculation of *L. lepideus*.

was identified using Stalpers (1978) 'Key to the identification of wood inhabiting aphyllophorales in pure culture' as *Sistotrema brinkmannii*.

Supplementary experiments were set up in which interactions between this fungus and both *Trichoderma* and *L. lepideus* (FPRL 7F) were monitored. The test method used was identical to the one employed for the interactive studies in Chapter 2. The *Sistotrema* was completely overgrown by the *Trichoderma* which appeared to induce a state of fungistasis in the former as it advanced over it. This type of reaction was classified previously as intermingling. When *Sistotrema* was tested against *L. lepideus* however a replacement type reaction resulted. On contact with the *Sistotrema* the mycelium of *L. lepideus* was lysed with subsequent release of red pigment into the media, further advance of the *Sistotrema* resulted in the complete overgrowth and accompanying replacement of the *L. lepideus*.

When the five poles in this category which were not sectioned were sampled in March 1983, two were found to contain *Trichoderma* but no *L. lepideus*, one contained *L. lepideus* but no *Trichoderma* and the other two contained both organisms.

(iv) Effect of subsequent inoculation with Binab FYT pellets on the colonisation of poles by *L. lepideus*

L. lepideus presence had been confirmed in all ten poles prior to their inoculation with Binab FYT pellets, on sectioning however only five of the ten poles were found to contain a viable *L. lepideus* infection. Of the ten poles which were extensively sectioned eight contained *Trichoderma* and five contained *L. lepideus*. Four poles contained *Trichoderma* but no *L. lepideus*, four poles contained both the control and decay fungi, one pole contained *L. lepideus* but no *Trichoderma* and one pole contained neither fungus. This latter pole was found however, on sectioning, to contain no uncreosoted wood. The creosote appeared to have permeated throughout the wood thus limiting colonisation by either *Trichoderma* or *L. lepideus*. Very few organisms were isolated from this stub with the creosote tolerant fungus *Cladosporium resinae* present in 51.5% of the isolates. This pole is an oddity and thus the results obtained from it can be disregarded when assessing the effectiveness of the biological control organisms.

The distribution of *Trichoderma*, *L. lepideus* and organisms resident in the poles are presented in Appendix IV (1-10). These show that in those poles from which both *Trichoderma* and *L. lepideus* were isolated the spread

of *Trichoderma* appears to be expanding from the groundline inoculation point but is still bordered by *L. lepideus* (see pole XD3 in Appendix IV(3)). This may indicate that the control fungus is slowly colonising the area previously occupied by the decay fungus since the spread of *L. lepideus* must have originated from this inoculation point.

As in all the previous poles large quantities of resident organisms were isolated from the poles.

The quantity and spread of *Trichoderma* was less than that found in either group 1 or group 3 however this may be entirely due to the shorter incubation period. The distribution diagrams appear to show no direct relationship between the levels of *Trichoderma* and *L. lepideus* however as before they indicate that a relationship between the level of colonisation by *Trichoderma* and the amount of resident organisms already present does exist.

Percentage isolation figures for *Trichoderma*, *L. lepideus* and resident organisms at each sampling level within the poles were calculated and are presented in Table 7.4. The percentage isolation of *Trichoderma* varied widely among the eight stubs in which it was found ranging from 1% to 26.6% and the percentage isolation of *L. lepideus* ranged from 3.4% to 41.1% in the five poles from which it was recovered.

(a)

Sampling Levels	Pole numbers									
	XF4	XD2	XD3	XF1	XC1	XF3	XF5	XF2	XD1	XC2
E^+	0	0	0	0	0	0	7.1	22.2	0	0
D^+	0	0	8.0	3.6	0	0	0	25.0	0	0
C^+	0	0	20.0	17.9	2.5	3.1	0	24.0	0	0
B^+	0	0	27.8	22.6	4.7	14.5	4.0	40.7	0	0
A^+	0	0	34.0	24.6	8.3	7.0	2.0	28.3	0	1.6
Groundline inoculation level 0	0	0	33.3	22.0	12.3	11.7	4.0	31.5	4.3	6.8
A^-	0	0	26.7	6.9	11.7	4.7	10.2	22.4	0	13.8
B^-	0	0	28.0	3.5	0	0	24.5	29.1	0	6.2
C^-	0	0	17.9	3.4	0	0	6.9	20.8	0	0
D^-	0	0	30.8	0	0	0	6.9	19.2	0	0
E^-	0	0	25.9	0	0	0	7.1	10.0	0	0
Total % isolation for all levels	0	0	25.3	11.6	4.9	4.8	7.3	26.5	0.6	3.6
Total number of samples	627	552	430	449	637	482	423	430	542	507

(b)

E^+	0	2.8	0	4.0	45.0	48.3	0	0	0	0
D^+	0	3.2	36.0	7.2	41.0	34.4	0	0	0	0
C^+	0	0	37.5	3.6	42.5	12.4	0	0	0	0
B^+	0	0	25.9	1.9	42.3	38.2	0	0	0	0
A^+	0	2.9	18.9	1.8	36.9	33.3	0	0	0	0
Groundline inoculation level 0	0	2.9	22.2	1.7	36.9	28.3	0	0	0	0
A^-	0	1.5	35.7	6.9	35.1	20.3	0	0	0	0
B^-	0	4.2	35.1	8.8	45.4	31.3	0	0	0	0
C^-	0	11.4	46.4	13.8	44.4	29.0	0	0	0	0
D^-	0	8.3	38.5	20.0	43.9	27.5	0	0	0	0
E^-	0	6.5	44.4	8.0	48.6	27.5	0	0	0	0
Total % isolation for all levels	0	3.4	30.0	6.2	41.1	29.9	0	0	0	0

(c)

E^+	33.3	47.2	92.3	88.0	37.5	44.8	71.4	37.0	90.9	35.5
D^+	44.4	54.8	48.0	82.1	20.5	50.0	92.9	41.7	93.5	66.7
C^+	52.9	81.8	20.0	78.6	30.0	6.9	96.6	52.0	97.1	62.2
B^+	64.6	83.3	40.7	67.9	18.8	38.2	96.0	31.5	98.5	72.7
A^+	75.7	88.6	43.4	71.9	25.0	36.8	92.2	64.2	97.1	91.9
Groundline inoculation level 0	89.5	91.3	40.7	76.3	24.6	51.7	84.3	57.4	95.7	83.1
A^-	64.6	85.3	19.6	74.1	33.8	59.4	79.6	51.7	100	58.5
B^-	73.8	77.4	21.0	75.4	26.0	50.0	64.2	47.3	100	64.6
C^-	63.4	68.6	17.9	75.8	27.8	64.5	93.1	50.0	100	67.7
D^-	55.8	66.7	15.4	50.0	21.9	62.1	88.9	42.3	100	48.5
E^-	54.8	67.7	25.9	92.0	16.2	69.0	85.7	73.3	96.9	53.5
Total % isolation for all levels	65.1	77.5	34.2	74.6	25.6	52.3	85.1	50.2	97.4	67.1

Table 7.4 Percentage isolation of (a) *Trichoderma*, (b) *L. lepideus* and (c) resident organisms from creosoted poles inoculated with *L. lepideus* followed by the inoculation of Binab FYT pellets.

As in the previous group of poles the spread of *L. lepideus* is greater below rather than above the groundline inoculation point with isolation figures of 48.3% and 41.1% respectively. Spread of *Trichoderma* above groundline (41.5%) however is similar to that found below groundline (39.1%).

Of the five poles in this group which were not sectioned, three have given a positive *L. lepideus* isolation and another a positive *Trichoderma* isolation during routine sampling in September 1982 and March 1983.

The range of resident fungi found during sectioning of poles was almost identical to that recorded during sampling of on line poles. Table 7.5 shows the frequency of isolation of pole resident organisms from the 41 sectioned pole stubs. The proportions of the various fungal types are similar to that found in the standing poles.

7.4 DISCUSSION

Various techniques could have been employed to examine the interactions between immunizing commensals and *L. lepideus* in *situ* in the interiors of creosoted distribution poles and may be categorised into two groups, either microscopic or microbiological examination.

Organism	Percentage isolation
Cladosporium resinae	25.3
Bacterial spp.	9.6
Fusarium spp.	7.4
Penicillium spp.	2.7
Paecilomyces variotii	2.4
Sistotrema brinkmannii	2.1
Phialophora spp.	0.8
Mucor sp.	0.3
Others	3.6

Table 7.5 Frequency of isolation (%) of resident pole fungi recovered from 41 sectioned stubs.

Microscopic techniques involve the examination of thin wood sections removed from the interaction interface between the two organisms. The similarity in dimension of the mycelia of *Trichoderma* and that of *L. lepideus* however made differentiation between the two fungi difficult to detect especially when some of the mycelium may be in a lysed state. Other techniques which may have been used included one recently developed at the Forest Products Laboratory at Princes Risborough which involves the use of pH indicators to indicate areas of fungal colonisation in wood. This technique was tested to see if it could be used to delineate the spread of *L. lepideus* and *Trichoderma* without having to totally section the poles. The method involved spraying cut surfaces of wood with indicator dyes which change colour due to pH changes in the wood produced during fungal colonisation. This proved ineffective however due to the creosote treatment which changes pH balances so critical to the success of this technique. However the requirements of the project demanded that the spread and interaction of decay and control fungi be measured and because of this microbiological examination was employed. The disadvantage with this technique however was that in order to map the spread of the two organisms a large number of replicates were required.

The results presented in this chapter showed conclusively that although *Trichoderma* was not naturally

present it was established in all the poles inoculated with Binab FYT pellets which were sectioned. At no point was *Scytalidium* isolated from any of the poles inoculated with the Binab pellets. *Trichoderma* was also isolated from the one pole from which its presence had not been established during routine sampling of on line poles. This might suggest that the technique used for sampling on line poles was most likely to minimise the figures for *Trichoderma* establishment which may well be nearer 100% rather than the 90-94% recorded in Chapter 6. In order to be certain of isolating any *Trichoderma* present in standing poles 3-4 well spaced cores may well have to be removed. This may not be practical as extensive core removal obviously reduces the strength properties of the poles.

Although *Trichoderma* was established in all the poles the frequency of isolation and the spread of the organism from inoculation with two pellets was variable and most importantly appeared to be influenced by the presence of resident pole organisms. *Trichoderma* was not isolated from wood occupied by resident fungi and at the very least its rate of colonisation of such wood appeared to be drastically reduced. This is in direct contrast to the results of agar interaction studies carried out between *Trichoderma* and common pole resident organisms (Chapter 2) during which *Trichoderma* quickly overgrew its

competitors without causing their death. This apparent anomaly may be entirely due to the lack of nutrients present in wood. Both *Trichoderma* and the non decay producing resident fungi are generally considered to grow in wood by utilising the small quantity of soluble nutrients left after drying of the green timber. Since the pole resident fungi were present prior to the invasion of the *Trichoderma* these organisms might have previously consumed these nutrients thus prohibiting the establishment of the control fungus. The ability of *Trichoderma* to produce antibiotics and volatiles on which its competitive qualities are dependent must also be in doubt in an environment devoid of nutrients. This lack of colonisation by *Trichoderma* illustrates Bruehl's (1975) findings that possession of a substrate is "nine points of the law". The range and occurrence of resident pole organisms presented here are only those isolated using malt extract agar and the use of other media may well result in completely different isolations.

Trichoderma spread did not appear to be influenced by the moisture gradients found in the pole stubs since its distribution was the same above and below the groundline and the poles were different in moisture contents (see Chapter 5). Since the viability of the pellets were tested prior to inoculation the wide range in the percentage isolation of *Trichoderma* cannot be put down to differences

in the level of inoculum. It is probable however that the spread of the control fungus would be improved if Binab FYT pellets were inoculated at more than one point around the circumference of the pole.

The spread of *L. lepideus* does not appear to be influenced to the same extent as that of *Trichoderma* by the presence of resident fungi in the poles. This is reasonable however since *L. lepideus* can break down the cellulose content of wood and therefore lack of nutrients may not prevent the basidiomycete from colonising the poles even when previously occupied by non decay resident fungi. This is again the reverse of the agar interactions observed between these fungi and emphasises the importance of evaluation of control organisms' competitive abilities in a field environment.

The spread of the decay fungus indicated that *L. lepideus* has no preference for sapwood or heartwood and that it colonised both. This result disagrees with the findings of the Midlands Electricity Board (Anonymous 1971) who recorded that decay was predominant in the untreated sapwood region of the poles and that the heartwood was more resistant to attack. It may be that rot is more predominant in this region purely because spores of the fungus, which gain entry into the pole via cracks, are most likely to be deposited in this region. Furthermore

since the percentage isolation of *1. lepideus* was always greater below the groundline it would appear that the growth of the decay fungus was dependent on the higher moisture levels found there. This may explain why the majority of poles fail within ± 1 foot of the groundline and may also determine the pattern of decay produced in the poles.

Although *L. lepideus* had been present in some poles for approximately 18 months and had colonised up to 40% of the groundline region on no occasion had the fungus totally decayed the wood substance. This would suggest that decay fungi may be present in wood over extensive areas prior to detection and that the 'hammer test' used by the electrical authorities to detect decay presence may be ineffectual in detecting early stages of decay. This latter method detects decay only when cavities have been produced and it could be extrapolated from the results of this study that viable colonisation by *L. lepideus* may have taken place in excess of 2 years prior to cavity formation. More important is the fact that by the time decay is detected using the hammer technique remedial treatments might not be applicable.

The isolation of a second basidiomycete from the poles was most interesting since none had been detected during the sampling of on line poles. *Sistotrema brinkmannii*

has been isolated from pine and spruce stumps (Kaarik and Rennerfelt, 1957) and these authors, after laboratory tests on wood blocks, showed that it was a 'weakly rotting fungus' which played little part in the breakdown of the tree stumps. John Savory (1983 personal communication) has described *S. brinkmannii* as colonising wood in a manner similar to that of *Trichoderma*. There would therefore seem to be little danger to the poles from decay by this fungus. More importantly perhaps from the viewpoint of biological control is the ability of this organism to overgrow and kill *L. lepideus* when grown in competition on malt agar. *S. brinkmannii* has been shown (Aufsess, 1976) to be antagonistic to both *F. annosus* and *Stereum sanguinolentum* in wood and it may be that the presence of this fungus in the pole stubs had resulted in some level of natural control of *L. lepideus*. The same natural control may also have been exerted by the *Fusarium* and *Penicillium* species which were isolated regularly from pole stubs since both of these fungi have been shown by agar studies to be able to overgrow and kill *L. lepideus*.

Subsequent interactive studies on agar between *S. brinkmannii* and *Trichoderma* resulted in the overgrowth of the basidiomycete, however no death or lysis was produced. This is identical to the reaction between *Trichoderma* and most non decay pole resident fungi. It

is however different from the replacement reactions produced when *Trichoderma* interacts with most other basidiomycetes as reported in Chapter 2 although it is the same as the interaction between *Trichoderma* and *Coriolus versicolor*.

When the results of isolations from poles inoculated with either Binab FYT pellets or *L. lepideus* were compared with isolations from poles inoculated with both the pellets and the decay fungus they show that pole treatment with Binab FYT pellets, either prior to or subsequent to *L. lepideus* inoculation, reduced the incidence of attack by the decay fungus. Although substantial numbers of poles still had *L. lepideus* present the decay fungus was either spacially separate from the control fungus or appeared to be under attack from the *Trichoderma*. No direct relationship existed between the amounts of *L. lepideus* and *Trichoderma* isolated from poles treated with both fungi however this may be entirely due to the restraint placed on the spread of *Trichoderma* by the high levels of resident fungi present in the poles. In order to overcome this problem it would be advisible to inoculate poles as early as possible before they become colonised with other organisms however as the poles used in this study were all less than 10 years old this would mean inoculating poles very soon after installation. Since *Trichoderma* has been shown to protect

wood blocks after the fungus had been killed and the blocks thoroughly leached this early life treatment may not be as impractical as it appears.

Although there is no direct evidence that *Trichoderma* is producing antibiotics *in vivo* against *L. lepideus* it is almost certainly emitting volatiles active against the decay fungus since volatile effects identical to those seen during experiments in Chapter 4 were repeated on plates used to isolate pole organisms. *L. lepideus* isolated from wood cores preferentially grew away from other *Trichoderma* containing cores situated in the same petri dish.

Although the results of this study are obtained from large numbers of samples these were removed from only 41 poles and thus their value is limited. However the incidence of *L. lepideus* is reduced by around 45% by both pre and post inoculation with Binab FYT pellets. No direct comparison can be drawn between percentage isolation of *L. lepideus* and *Trichoderma* in different poles because the incubation periods are not uniform however *L. lepideus* was isolated from only five of the ten poles in each of the 2 groups which were inoculated with FYT pellets compared with reisolation from nine of the ten controls. This is particularly encouraging in view of the following:

1) The short field exposure of between 12 and 18 months, longer exposure might result in total replacement of the decay fungus by *Trichoderma*.

2) Differences in biomass inocula. Control organisms were inoculated at one point in the pole and FYT pellets contained only spores and mycelial fragments of the immunizing commensals whereas a large established biomass of *L. lepideus* was inoculated at three positions. The natural mode of infection of *L. lepideus* in poles is via a single spore and thus the test system used was very severe indeed.

3) The method employed for calculating the percentage isolation of both *Trichoderma* and *L. lepideus* tends to minimise their spread. This is due to the circular shape of the poles and the sampling technique. More wood samples were removed from the outer circumference of the uncreosoted region of the pole (i.e. the region furthest from the inoculation point) than from the region in the centre of the pole where the fungi were inoculated thereby minimising the percentage isolation of the organisms which is calculated on the total number of samples removed. For instance *Trichoderma* may have a percentage isolation value of 65% at any one sampling level, even though the fungus may occupy 100% of the area near the inoculation point.

In summary the results presented in this chapter conclusively show

1) That spread of *Trichoderma* from a single inoculation of two Binab FYT pellets is extensive though it may be limited by the presence of other resident pole organisms

2) That spread of *L. lepideus* in poles is rapid and takes place without decay in the first instance. In consequence of which current decay detection systems may be ineffective.

3) That the colonisation of wood by *L. lepideus* may be prevented by either prior or subsequent inoculation of Binab FYT pellets.

CHAPTER 8

GENERAL DISCUSSION

The work described in this thesis was part of a CASE project and was undertaken with two major objectives in mind. The first was to examine the possibility of employing a biological control system as an alternative to the chemical treatments currently used for the remedial treatment against *L. lepideus* of creosoted distribution poles. The second objective was to monitor the success or otherwise of a commercially manufactured biological product, namely Binab FYT pellets, used to inoculate mature creosoted distribution poles.

Before a problem can be solved it must be firstly established that one exists. Cartwright and Findlay (1958) reported *L. lepideus* to be one of the major causes of decay in creosoted timber and the findings of King and Penn (1975 unpublished data) during sampling of creosoted distribution poles showed the same fungus to be the major decay causative organism. The results of this present study agree with the findings of earlier workers and *L. lepideus* was isolated regularly from samples removed from decayed poles. Only one other basidiomycete was occasionally isolated from the creosoted poles and this was identified as *S. brinkmannii* which does not produce significant levels of decay in wood.

Since biological control agencies are usually host specific the fact that *L. lepideus* is largely responsible for decay in creosoted poles suggests that it may be an ideal target for an effective immunizing commensal.

Although decay by *L. lepideus* was almost exclusively restricted to the groundline region of affected poles the same organism was found to be responsible for the decay which occurred in some pole tops. The results suggest that the spread of *L. lepideus* may be influenced by moisture gradients in the poles (preferential growth occurring towards wetter regions) and thus pole tops which are susceptible to attack by this organism may also have excessive moisture contents. This is likely to be the case in poles in the high rainfall regions of the West of Scotland where this type of decay is prevalent. As with any investigation of biological control systems the work undertaken in this thesis was carried out in an ordered sequence

1) The evaluation of control of *L. lepideus* on synthetic media in the laboratory.

2) The evaluation of control of *L. lepideus* in wood under laboratory conditions.

3) The evaluation of control of *L. lepideus* in standing creosote treated poles.

and the results of any one stage indicated whether further investigation was likely to be worthwhile.

The first stage of the investigation was undertaken to show whether the control organisms *Scytalidium* FY strain and Binab *Trichoderma* were capable of overgrowing and killing *L. lepideus* when grown on agar media under laboratory conditions (Chapter 2). The control fungi quickly achieved this at both 25°C and 10°C but not at 5°C. As an extension of the agar studies various interactions were also examined between the control organisms and non decay fungi isolated from routine sampling of creosoted distribution poles. These interactions always resulted in one of two reaction types, (a) the specific intermingling reaction of *Trichoderma*, by which that fungus overgrew pole resident fungi without causing their death or (b) the stalemate reaction specific to *Scytalidium* FY strain, in which neither the pole resident fungi nor the *Scytalidium* entered the areas occupied by the other. Interactions were also monitored between various pole resident fungi including *L. lepideus*. In most cases a stalemate reaction resulted however more interestingly pole isolates of *Penicillium*, *Fusarium*, *Scytalidium* and *Sistotrema brinkmannii* all replaced *L. lepideus* bringing about its death in a manner identical to that produced by Binab *Trichoderma*. This result, if repeated *in situ* in the poles, would suggest that some form of natural

control may occur which could in part explain the large variability in lifespan of creosoted distribution poles. Using similar interactive tests on agar plates the specificity of *Trichoderma* against a range of wood decay organisms including *F. annosus*, *C. versicolor*, *S. lacrimans* and *C. puteana* was also examined and the control fungus was shown to replace all except *C. versicolor*, interaction with which resulted in stalemate. This wide varying action of *Trichoderma* among wood decay organisms has interesting implications which warrant further study.

The natural progression of the investigation involved experimentation to monitor whether both *Scytalidium* FY strain and *Trichoderma* could protect wood from decay by *L. lepideus*. Since longevity of the control fungi once inoculated into creosoted poles was doubtful due to environmental and nutrient limitations the ability of control organisms to produce residual protection was also examined. The results (described in Chapter 3 and in Bruce and King, 1983) showed that pine and lime blocks pretreated with either *Scytalidium* or *Trichoderma* were resistant to decay by *L. lepideus* even when control organisms were killed and the wood blocks thoroughly leached prior to being exposed to the decay fungus.

The mechanism of action of *Scytalidium* FY strain is well established (Ricard and Bollen, 1968) and has been shown to be by antibiotic production. Klingstrom and Johansson (1973) have described antagonism via antibiotic production by *Scytalidium* isolates against decay fungi including *L. lepideus*.

Although *Trichoderma* has been employed in many biological control systems for the protection of plants from pathogens, prior to this study, no record was available to describe *Trichoderma's* mode of action against *L. lepideus*. Using broth culture techniques Binab *Trichoderma* was shown to produce soluble antibiotics which were active against *L. lepideus*. Employing techniques based on that of Dick and Hutchinson (1966) Binab *Trichoderma* was also shown to produce volatiles fungistatic as well as fungicidal against *L. lepideus* (Chapter 4 and Bruce, Austin and King, 1983). Although Dennis and Webster (1971b) had shown that *Trichoderma* species produced volatiles active against a wide range of fungi including basidiomycetes, only Russian workers Bilai (1956) and Koshanov (1962) (quoted in Dennis and Webster, 1971b) have previously shown fungicidal effects by this fungus. The studies described in this thesis show for the first time that *Trichoderma* species can produce volatiles which have fungicidal effects on *L. lepideus*.

Once control of *L. lepideus* had been established under laboratory conditions, field studies were undertaken to examine the efficiency of *Trichoderma* and *Scytalidium* in poles inoculated with Binab FYT pellets. The monitoring of moisture and nutrient levels of poles on site was therefore most important in determining their suitability for growth of control fungi. Moisture contents of over 200 poles were found to range from 18% to a state of total water saturation. *Trichoderma* however was found to grow throughout the whole range with the exception of the occasional totally saturated pole. Little nutrient transfer occurred from soil to poles irrespective of the moisture status of the wood (Chapter 5 and King, Mowe, Bruce and Smith, 1981). Although mean nitrogen contents of the soils at the three sites varied widely mean nitrogen contents of the poles were the same at each site. Thus, although other chemical compounds may be transferred to wood there appears to be little transfer of growth limiting nutrients especially nitrogen. Survival and spread of the control fungi are therefore limited by the quantity of soluble nutrients present in the wood and those amounts added during remedial treatment of the poles. These obvious nutrient restraints however do not limit the establishment of *Trichoderma* from inoculation of creosoted poles with Binab FYT pellets. *Trichoderma* was reisolated from 90-94% of poles at three sites although the

isolation technique employed was liable to minimise the percentage reisolation. No differences existed between the sites with regard to the frequency of *Trichoderma* establishment even though the sites differed with respect to mean average rainfall levels, temperatures, soil conditions and years in which poles were installed. Pole age and presence of decay had no deleterious effect upon the frequency of *Trichoderma* establishment from FYT pellet inoculation. The control fungus also became established in 'cobra treated' poles although the rate at which it spread through such poles was reduced compared with poles which had undergone no previous remedial treatment (Chapter 6).

The extent to which *Trichoderma* colonised creosote treated poles from a single inoculation of two FYT pellets and interactions in such poles between *Trichoderma* and *L. lepideus* were examined in Chapter 7. Evidence was produced from these studies which conclusively showed that the control fungus was capable of inhibiting *L. lepideus in situ* in creosoted poles. Sectioning and replicate sampling of stubs removed from the groundline regions of poles was chosen as the most practical experimental technique for this purpose. A total of 22,920 samples were plated from the groundline regions of 41 poles and the results showed that,

(a) *Trichoderma* was established in all the poles inoculated with Binab FYT pellets. Its spread was variable and appeared to be directly related to the quantity of resident fungi present in the stubs.

(b) No *Scytalidium* was recovered from either FYT pellet inoculated poles or indeed the pellets themselves.

(c) In contrast to agar studies, *Trichoderma* could not readily intermingle with the pole resident organisms which underlines the well established limitations of using studies on synthetic media. (Results of competition in wood between *L. lepideus* and pole resident fungi were also found to differ from those witnessed during agar studies.)

(d) The spread of *L. lepideus* in poles appeared to be influenced by moisture gradients.

(e) Large quantities of *L. lepideus* may be present in viable form in creosote treated poles for long periods prior to the production of decay cavities.

(f) Inoculation of poles with Binab FYT pellets reduces the incidence of decay produced by either prior or subsequent inoculation with *L. lepideus*. The success of the treatment however appeared again to be

linked to the levels of pole resident fungi.

(g) The studies were biased in favour of the decay fungus as the inoculum of *L. lepideus* used to inoculate the poles was far greater than that of the control organisms present in the Binab FYT pellets.

An important aim of this investigation was to provide the electrical authorities with an appraisal of the feasibility of using the Binab FYT pellets in practice. It was found that the pellets were easy to apply; the inoculation process was quick (approximately 5 minutes per pole) and required only one person; pellet viability remained high provided that the pellets were stored at 5°C; the cost of treatment was low compared with currently used remedial treatments, which cost is closely related to the ease of application and the small amount of manpower required. *Scytalidium* FY strain was never isolated from any of the poles inoculated with Binab FYT pellets. This may be beneficial however since the long history of *Trichoderma* usage in biological control systems has helped to establish that the fungus is non-toxic to the handlers and could thus be applied by the board's own employees unlike the toxic chemicals currently used.

Although *Scytalidium* FY strain has been shown to actively control *L. lepideus* both on agar and in wood blocks in the laboratory (Chapters 2 and 3), at no time

during the project was the fungus isolated either from the poles or on agar from the pellets. The results of studies described in Chapter 4 showed that the growth of *Scytalidium* FY strain was found to be inhibited by both volatiles and antibiotics produced by *Trichoderma*. This allied to the faster growth of the latter would seem to indicate that at least in the short term *Scytalidium* will not become established in poles inoculated with FYT pellets.

The *Trichoderma* strains present in the pellets are capable of reducing the incidence of decay by *L. lepideus*. These results were specific to the *Trichoderma* strains used however and may not be produced by all *Trichoderma* strains. The ability to produce antibiotics and volatiles active against *L. lepideus* may be vitally important to the success of the Binab product. Dennis and Webster (1971a and b) have shown that very wide variability exists between the members of the genus *Trichoderma* with respect to antibiotic and volatile production and it is therefore apparent that some form of quality control must be undertaken to ensure that the composition of the *Trichoderma* strains in the pellets remain such that maximum control of *L. lepideus* is continually achieved.

The effectiveness of the Binab *Trichoderma* strains may be limited, at least in the short term, by their failure to spread efficiently throughout those areas of the

poles which are occupied by other resident organisms. This defect might be overcome by inoculating poles soon after installation i.e. before colonisation by resident fungi had taken place, or by further strain selection.

The duration of active growth of *Trichoderma* in creosoted poles is unknown thus the extent of pole protection has not been established. However its ability to produce residual protection after its death has been shown (Chapter 3) and if such protection is reproduced throughout the groundline regions of creosoted poles *Trichoderma* may be able to protect poles even after active growth and colonisation has ceased. If this residual protection is not produced in poles however, it is possible that the low cost of the treatment might permit reapplication at more frequent intervals e.g. 3-5 years and yet still be as cost efficient as the currently used remedial treatments. However the effect of reinoculation of *Trichoderma* has not been established.

The efficiency of the process studied during this project could almost certainly be increased if the product was inoculated at more than one position around the circumference of the poles. The results (Chapter 7) suggest that *Trichoderma* inocula which are completely bordered by wood colonised by pole resident organisms are prevented from spreading to other areas of the pole even though those are uninhabited thus leaving them open to

attack from *L. lepideus*. Multiple inoculations may overcome this problem and help maximise the spread of the control fungus whereby increasing the efficiency of the process.

The results described in Chapter 7 show that the Binab product and inoculation used reduced the incidence of attack. These results were achieved from a relatively small number of poles and although they are most encouraging further studies on large pole populations would be required before comparisons could be drawn between the success of Binab FYT pellets and remedial treatments currently used by the electrical authorities. Further studies are also required to maximise the efficiency of the Binab product. This would include establishing the most effective formulation of the product, the ideal pole age for treatment and the most efficient inoculation process. In addition tests would also need to be undertaken to examine the effect if any of Binab FYT pellet treatment on the strength properties of the poles. Although laboratory techniques are available to measure strength losses produced in wood by fungal colonisation these are far removed from the breaking strength tests which would be required for creosote treated poles.

The fact that *L. lepideus* is the organism largely responsible for decay in creosoted poles, an environment with a limited range of natural inhabitants, indicates that it would be an ideal target for a host specific biological control agent. However three criteria would have to be satisfied by any such agent.

1) The control fungus should survive in the specific environment in which the host is active without causing damage to the substrate which is to be protected.

2) The control fungus should act against the target fungus in this environment.

3) There should be as little interference as possible from other resident organisms on the fine balance between target and control organisms.

Although Binab *Trichoderma* satisfies the first two criteria the presence of pole resident fungi e.g. *C. resinae* greatly reduce its effectiveness. The results of this investigation indicate however that some pole resident fungi also themselves inhibit or kill *L. lepideus* and since these organisms are naturally present in poles further research may well show that one or more of these could be more successful in the biological control of *L. lepideus*.

The work described in this thesis has examined the concept of using biological agents to control the internal decay in creosoted distribution poles. In the process, a biological product, the Binab FYT pellet, produced specifically for this purpose, has been evaluated for the Electrical Supply Industry. The results presented provide fundamental information on the biological control of *L. lepideus* by the immunizing commensals contained in Binab pellets both in the laboratory and in creosoted poles in the field and for the first time control mechanisms of *Trichoderma* against *L. lepideus* are demonstrated. A number of problems likely to be of importance in the application of biological control of wood in the field were identified. However it is clear from the findings described in this thesis that with further development biological control may be one other form of preservation which may be used to protect creosoted wood in service as distribution or transmission poles.

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APPENDICES

APPENDIX I

Diagrams showing the distribution of *Trichoderma* and pole resident organisms isolated from sections removed from the groundlines of 11 poles inoculated with Binab FYT pellets in June 1981.

(All poles were inoculated at the groundline and the diagrams represent the distribution as seen from an angle 10° above the plane on which the section sits.)

In the following diagrams the pole sections are positioned as in Figure 7.1(1A) with the dissection plane passing directly through the groundline inoculation point.

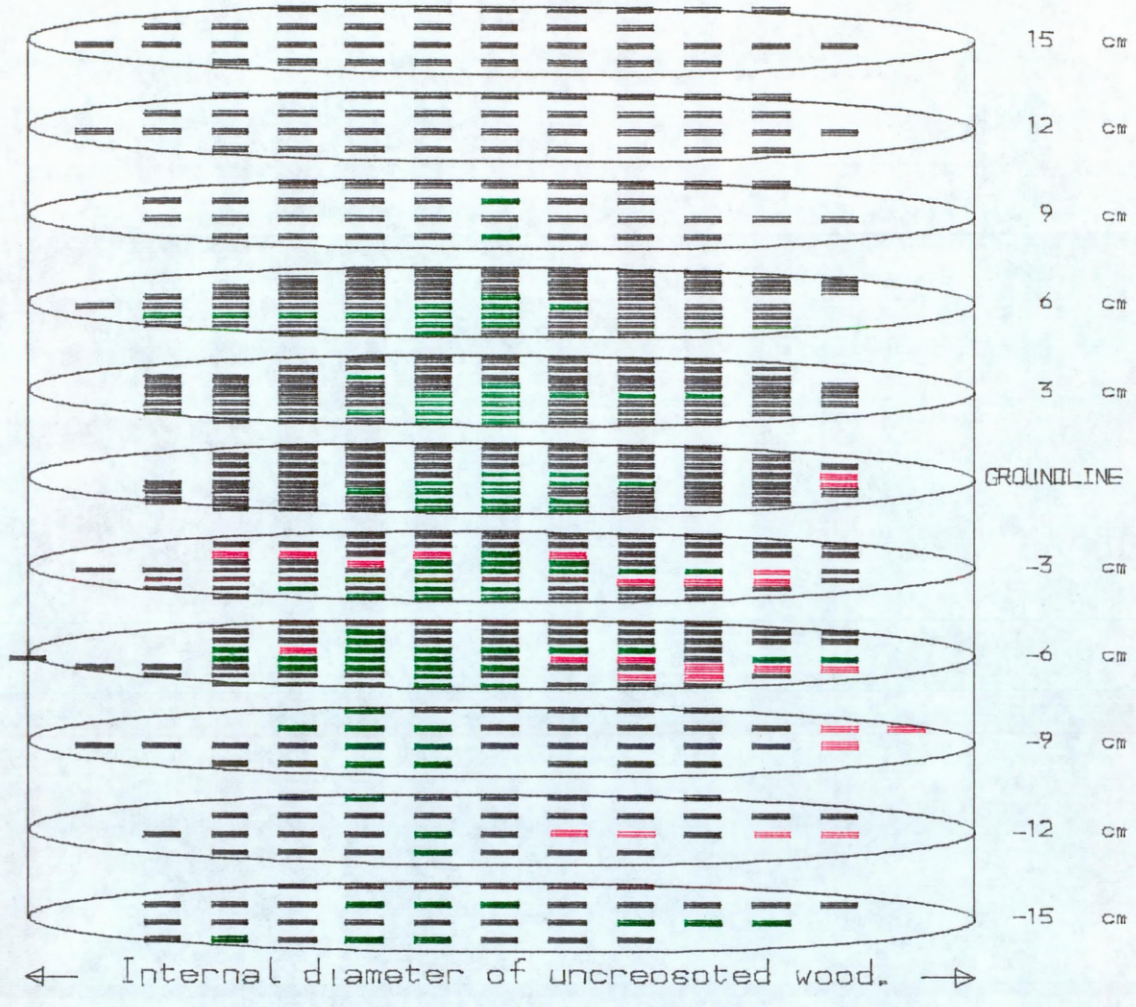
Sample levels at increasing heights above and below the groundline represented here as 3 cms → 15 cms and -3 cms →-15 cms correspond respectively to levels $A^+ \rightarrow E^+$ and $A^- \rightarrow E^-$ in Figure 7.1(2).

KEY

GREEN:- Trichoderma isolates.

RED :- Lentinus lepideus

BLACK:- Other resident pole organisms



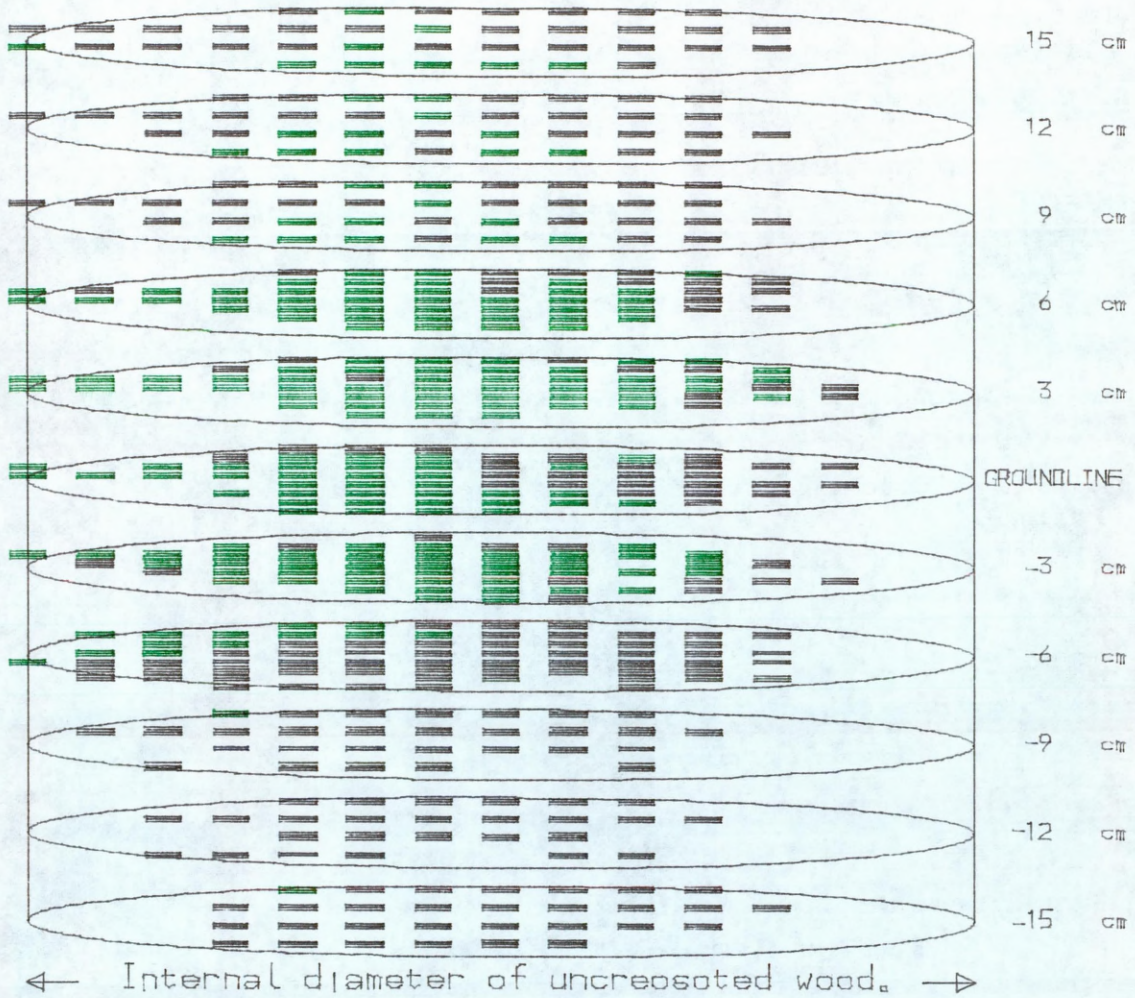
Pole number: XB5 Sectioning date: 22/11/82

FYT pellet inoculation date: 18/6/81

Lentinus inoculation date: NONE

KEY

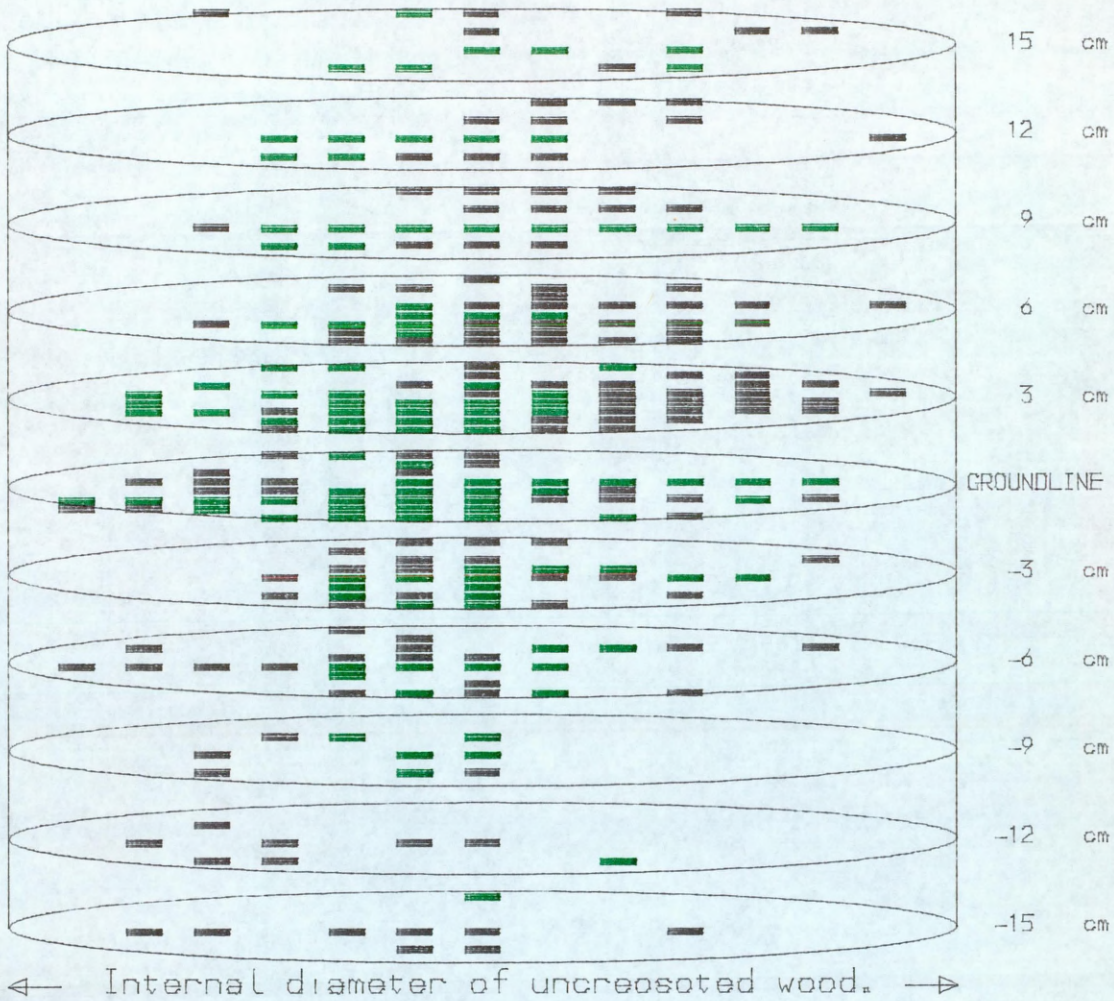
GREEN:- Trichoderma isolates.
RED :- Lentinus lepideus
BLACK:- Other resident pole organisms



Pole number: SCOT Sectioning date: 26/3/82
FYT pellet inoculation date: 12/8/81
Lentinus inoculation date: NONE

KEY

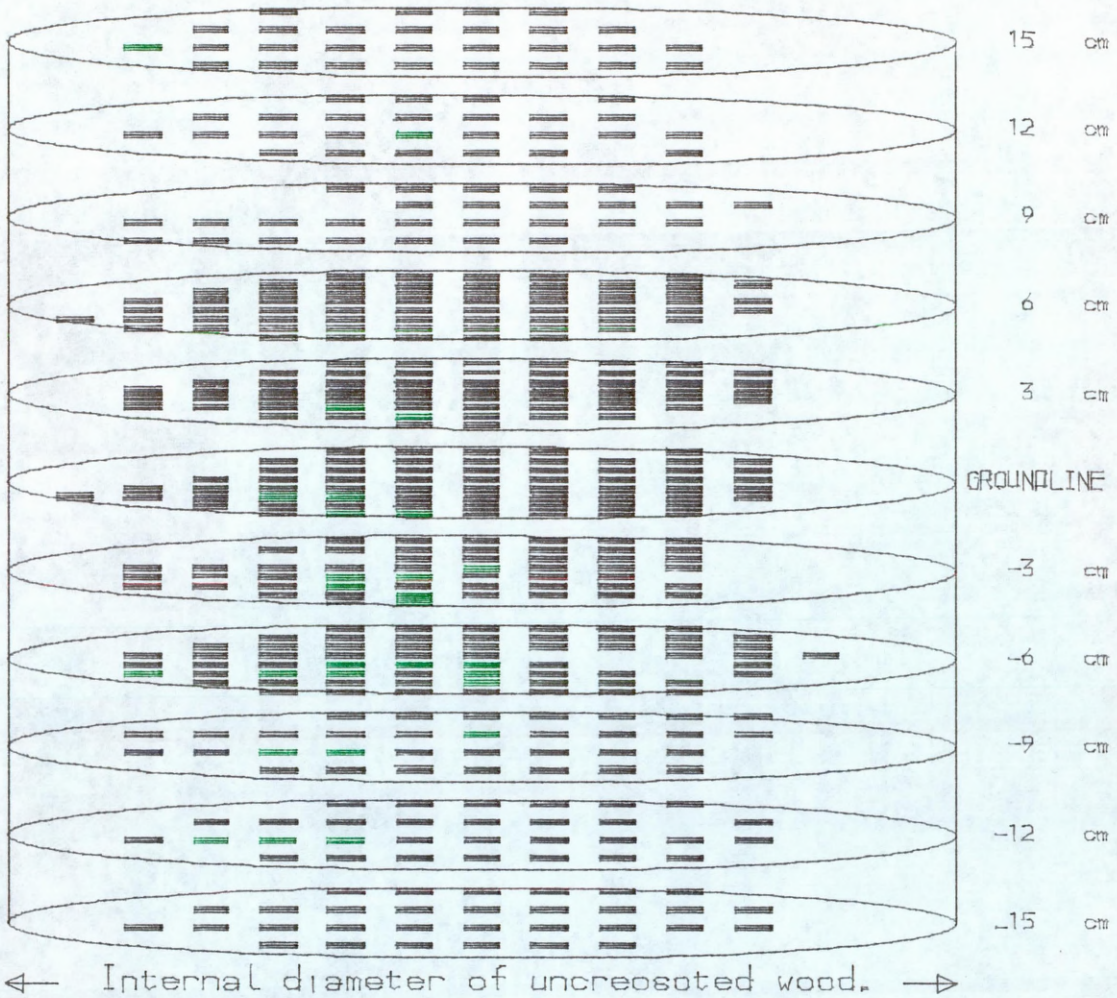
GREEN: - Trichoderma isolates.
RED : - Lentinus lepideus
BLACK: - Other resident pole organisms



Pole number: XS Sectioning date: 1/3/82
FYT pellet inoculation date: 18/6/81
Lentinus inoculation date: NONE

KEY

GREEN:- *Trichoderma* isolates.
RED :- *Lentinus lepideus*
BLACK:- Other resident pole organisms



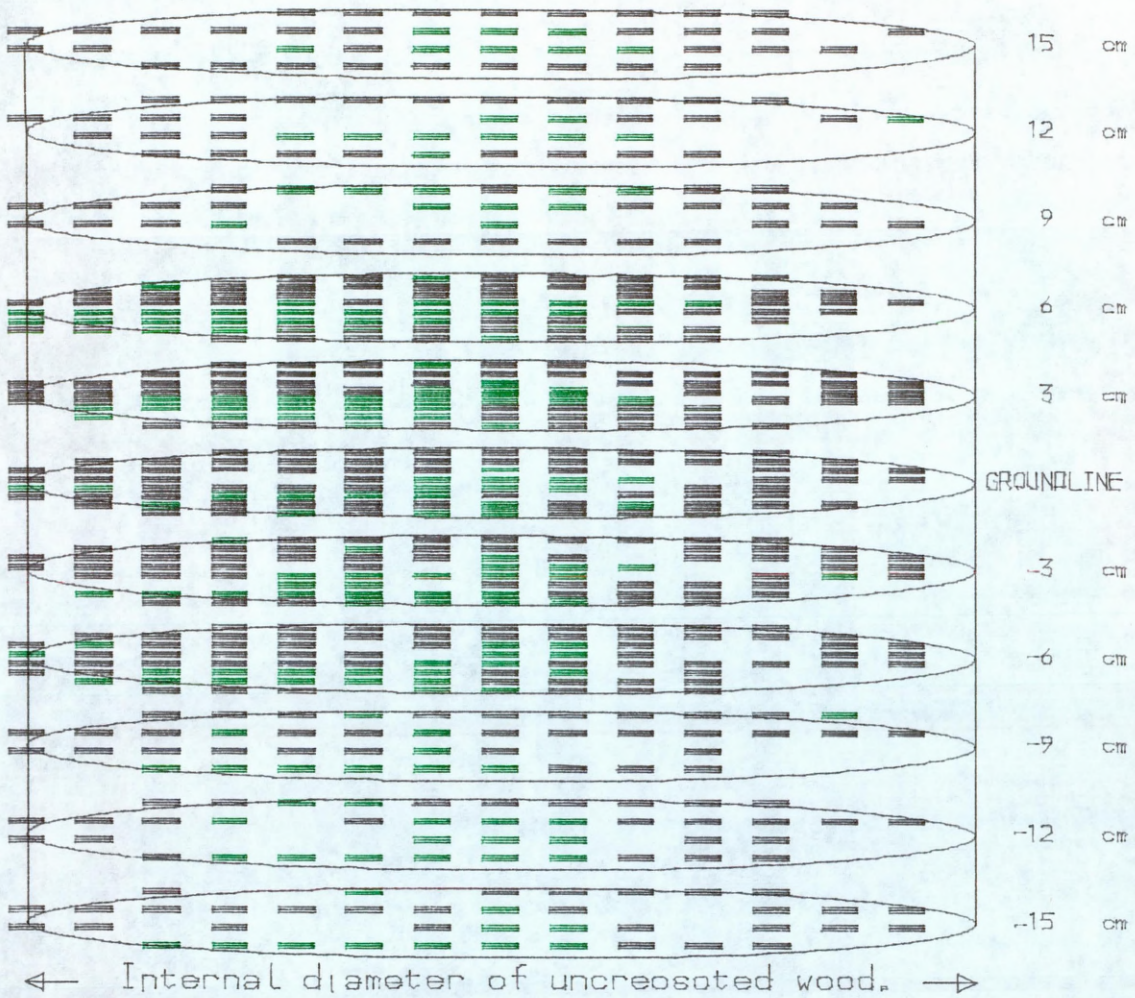
Pole number: XB3 Sectioning date: 29/11/82
FYT pellet inoculation date: 18/6/81
Lentinus inoculation date: NONE

KEY

GREEN:- Trichoderma isolates.

RED :- Lentinus lepideus

BLACK:- Other resident pole organisms



Pole number: XB6F Sectioning date: 8/4/82

FYT pellet inoculation date: 18/6/81

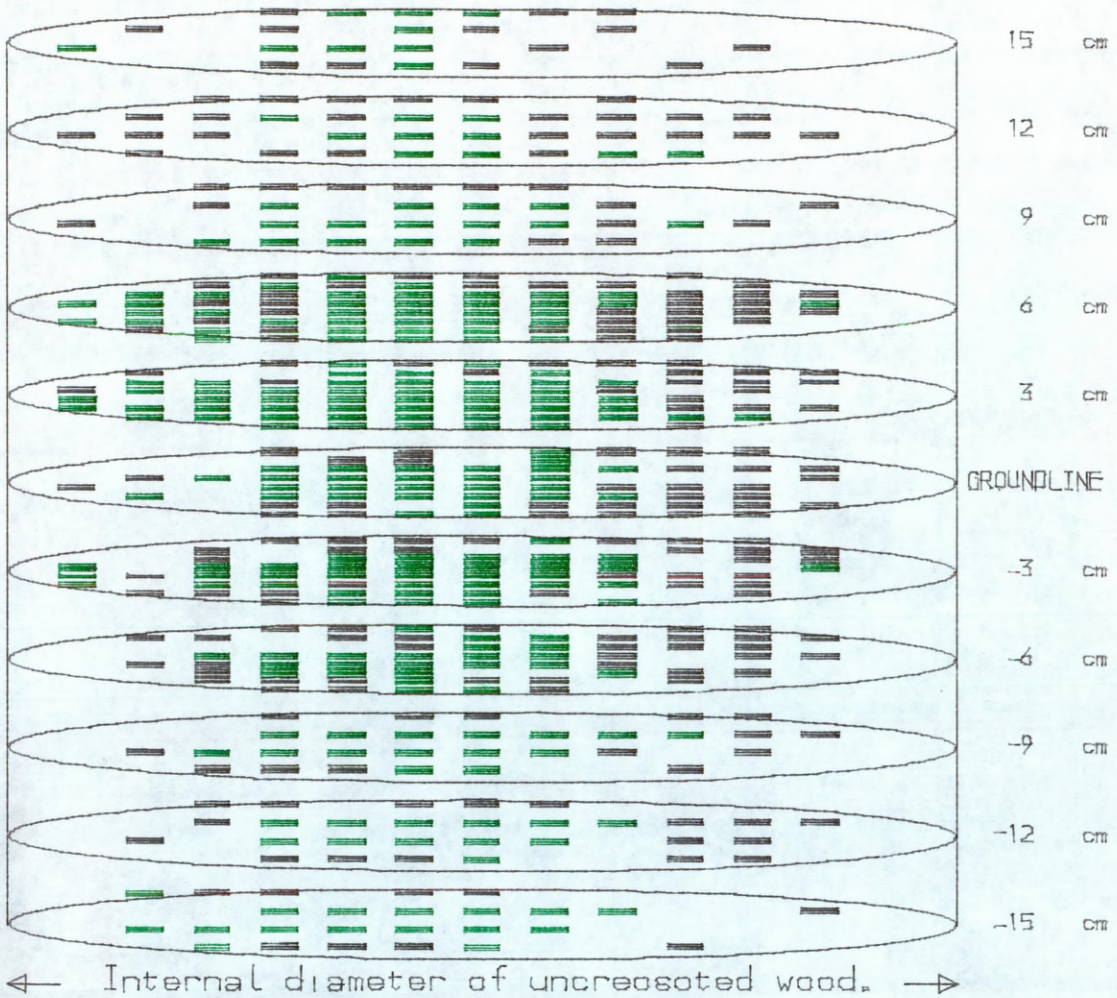
Lentinus inoculation date: NONE

KEY

GREEN:- Trichoderma isolates.

RED :- Lentinus lepideus

BLACK:- Other resident pole organisms



Pole number: XC3L Sectioning date: 13/4/82

FYT pellet inoculation date: 18/6/81

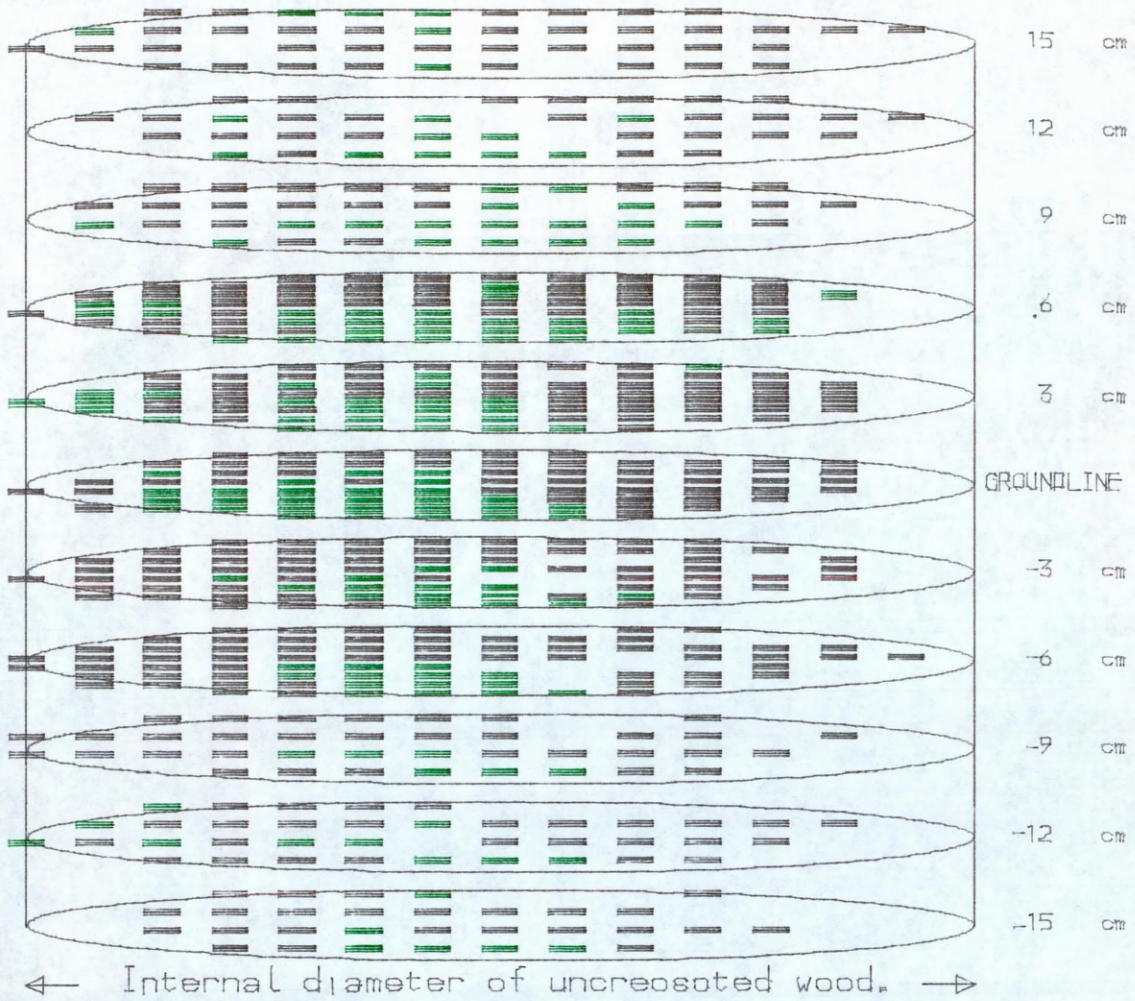
Lentinus inoculation date: NONE

KEY

GREEN:- Trichoderma isolates.

RED :- Lentinus lepideus

BLACK:- Other resident pole organisms



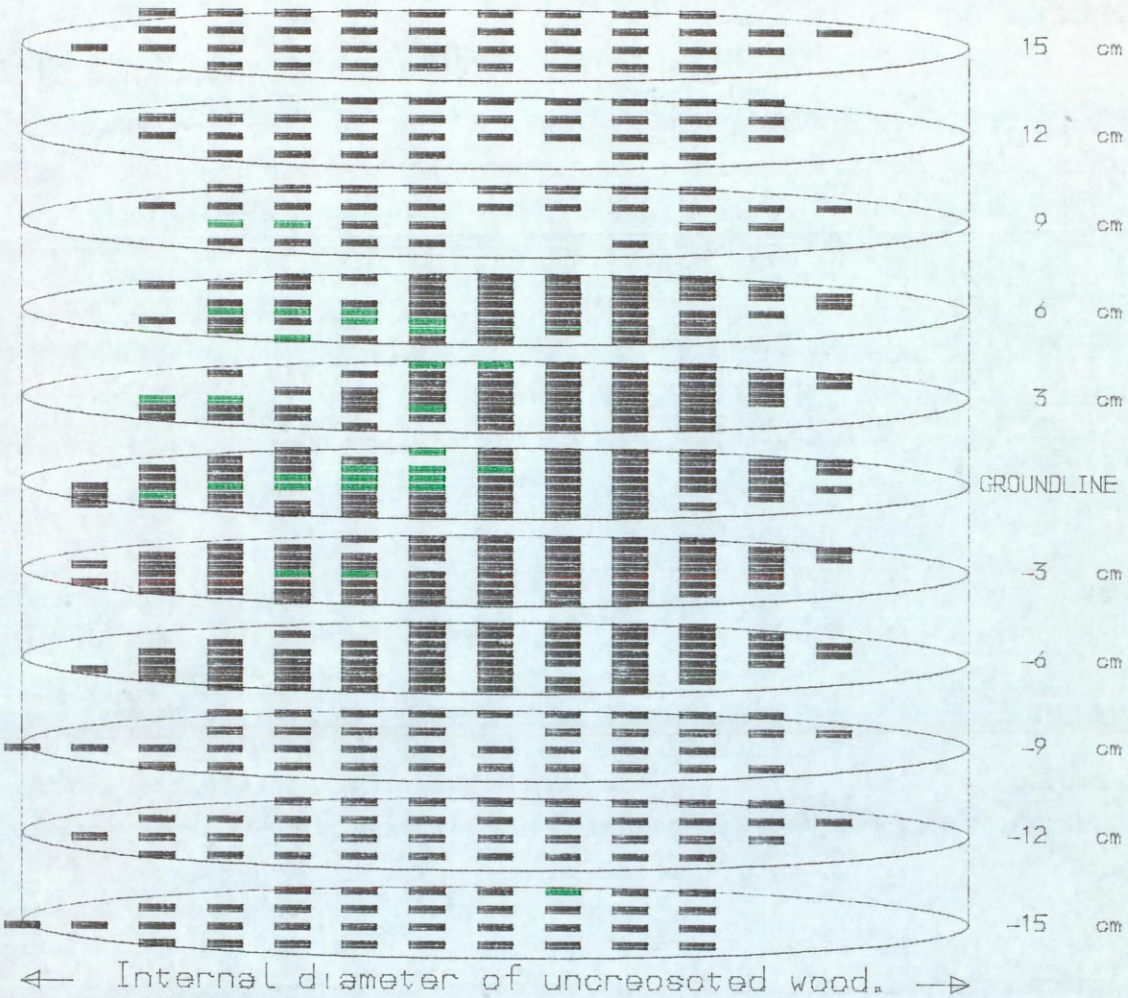
Pole number: D5F Sectioning date: 15/11/82

FYT pellet inoculation date: 18/6/81

Lentinus inoculation date: NONE

KEY

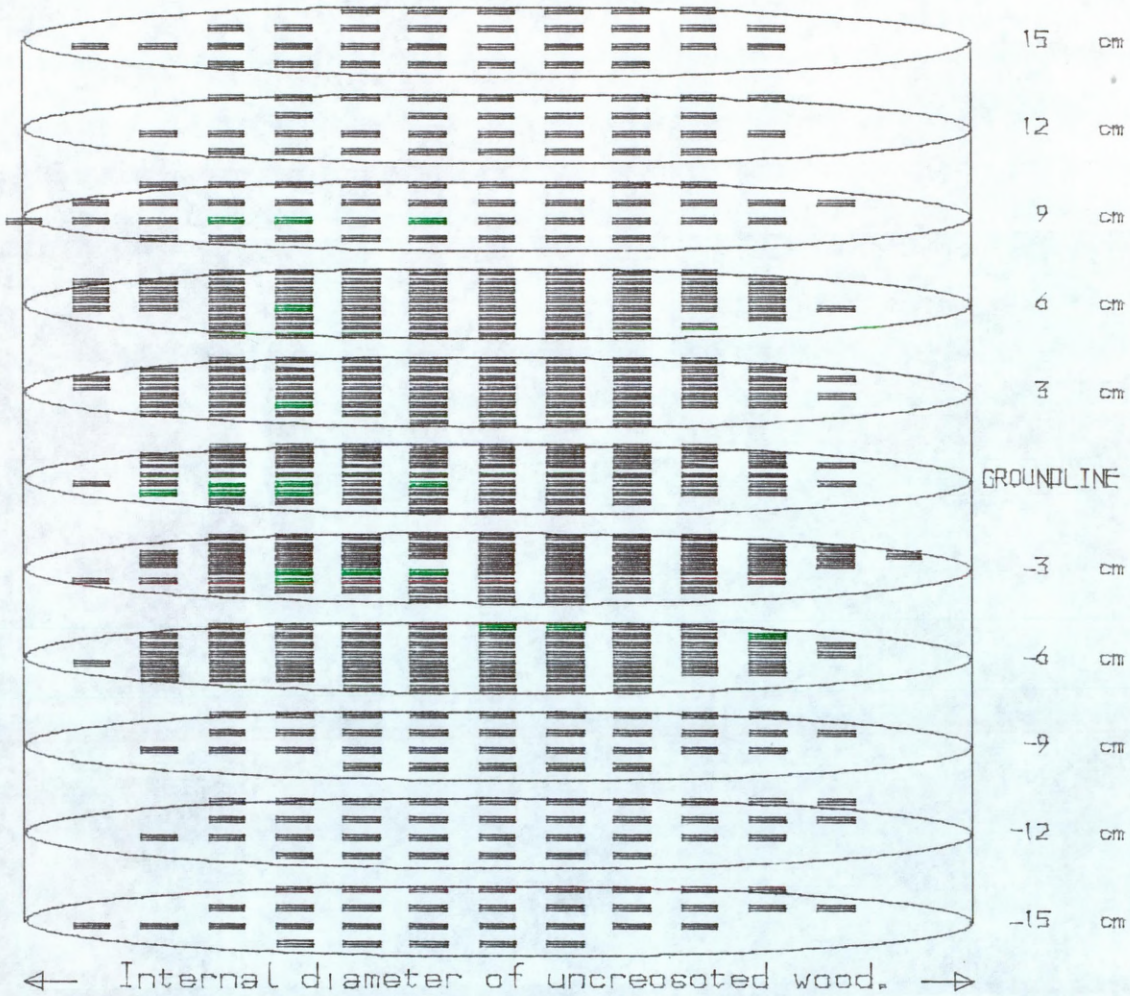
GREEN: - Trichoderma isolates.
RED : - Lentinus lepideus
BLACK: - Other resident pole organisms



Pole number: XD4 Sectioning date: 9/11/82
FYT pellet inoculation date: 18/6/81
Lentinus inoculation date: NONE

KEY

GREEN:- Trichoderma isolates.
RED :- Lentinus lepideus
BLACK:- Other resident pole organisms



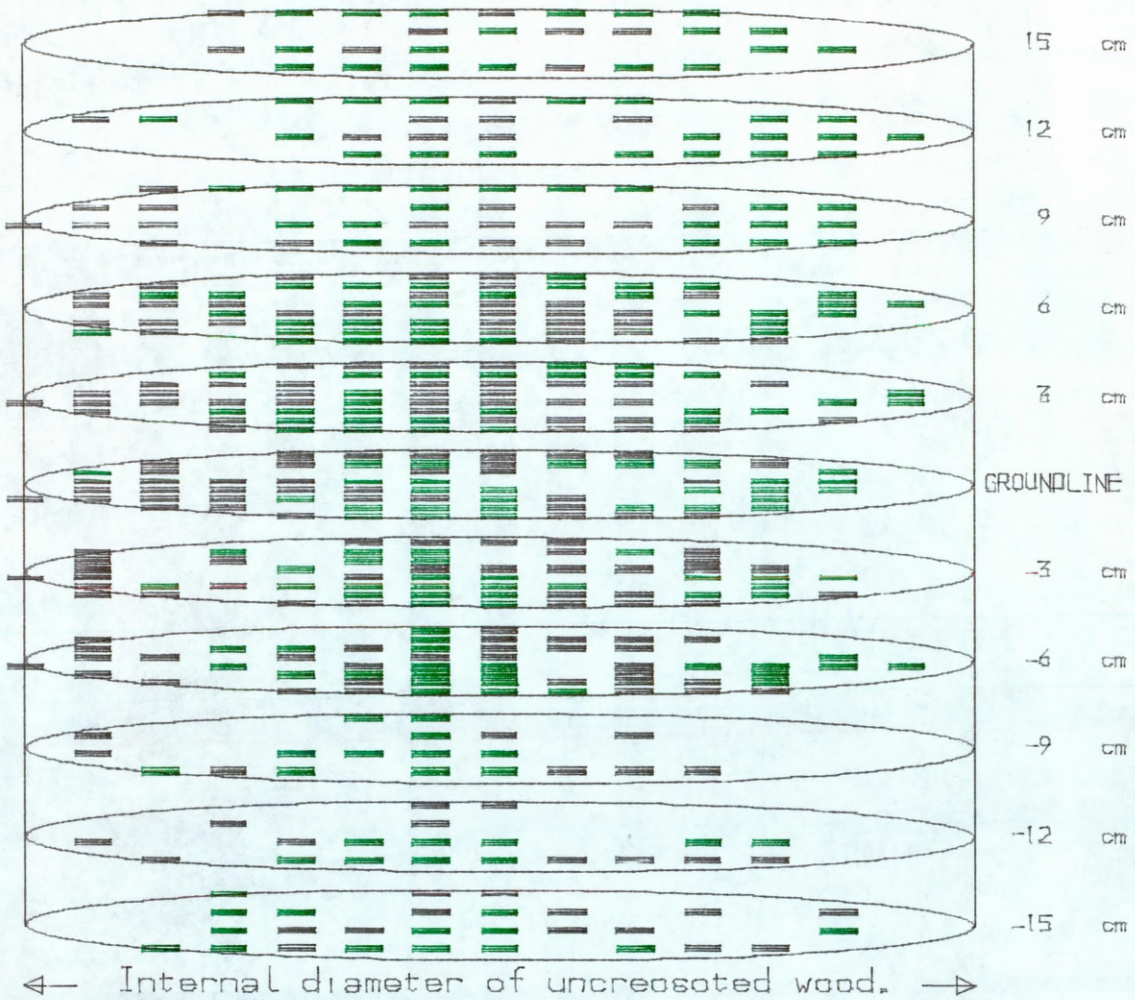
Pole number: XC3R Sectioning date: 6/12/82
FYT pellet inoculation date: 18/6/81
Lentinus inoculation date: NONE

KEY

GREEN:- Trichoderma isolates.

RED :- Lentinus lepideus

BLACK:- Other resident pole organisms



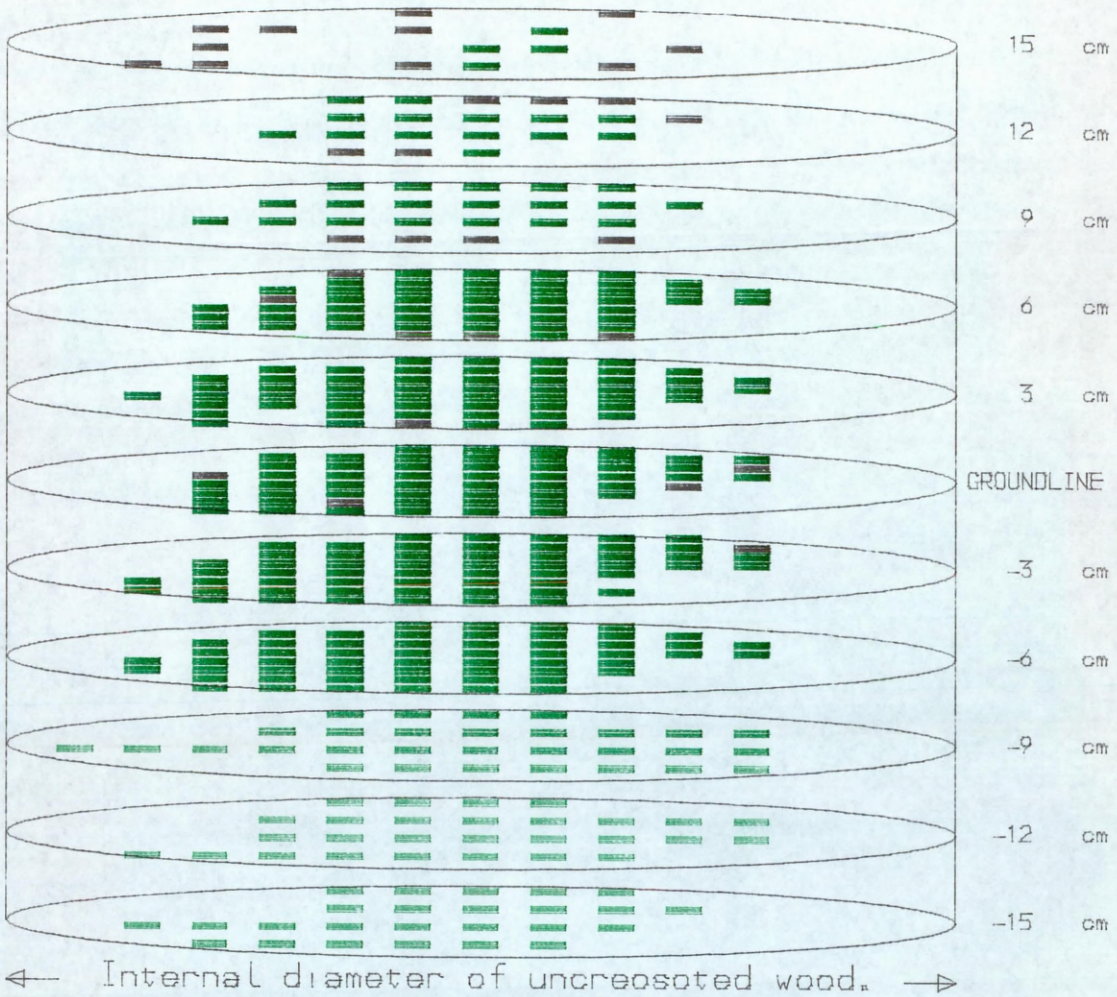
Pole number: XC4F Sectioning date: 3/4/82

FYT pellet inoculation date: 18/6/81

Lentinus inoculation date: NONE

KEY

GREEN = Trichoderma isolates.
RED = Lentinus lepideus
BLACK = Other resident pole organisms



Pole number: XC4B Sectioning date: 4/4/82
FYT pellet inoculation date: 18/6/81
Lentinus inoculation date: NONE

APPENDIX II

Diagrams showing the distribution of *Lentinus lepideus* and pole resident organisms isolated from sections removed from the groundlines of 10 poles inoculated artificially with *L. lepideus*.

(All poles were inoculated at the groundline and the diagrams represent the distribution as seen from an angle 10° above the plane on which the section sits.)

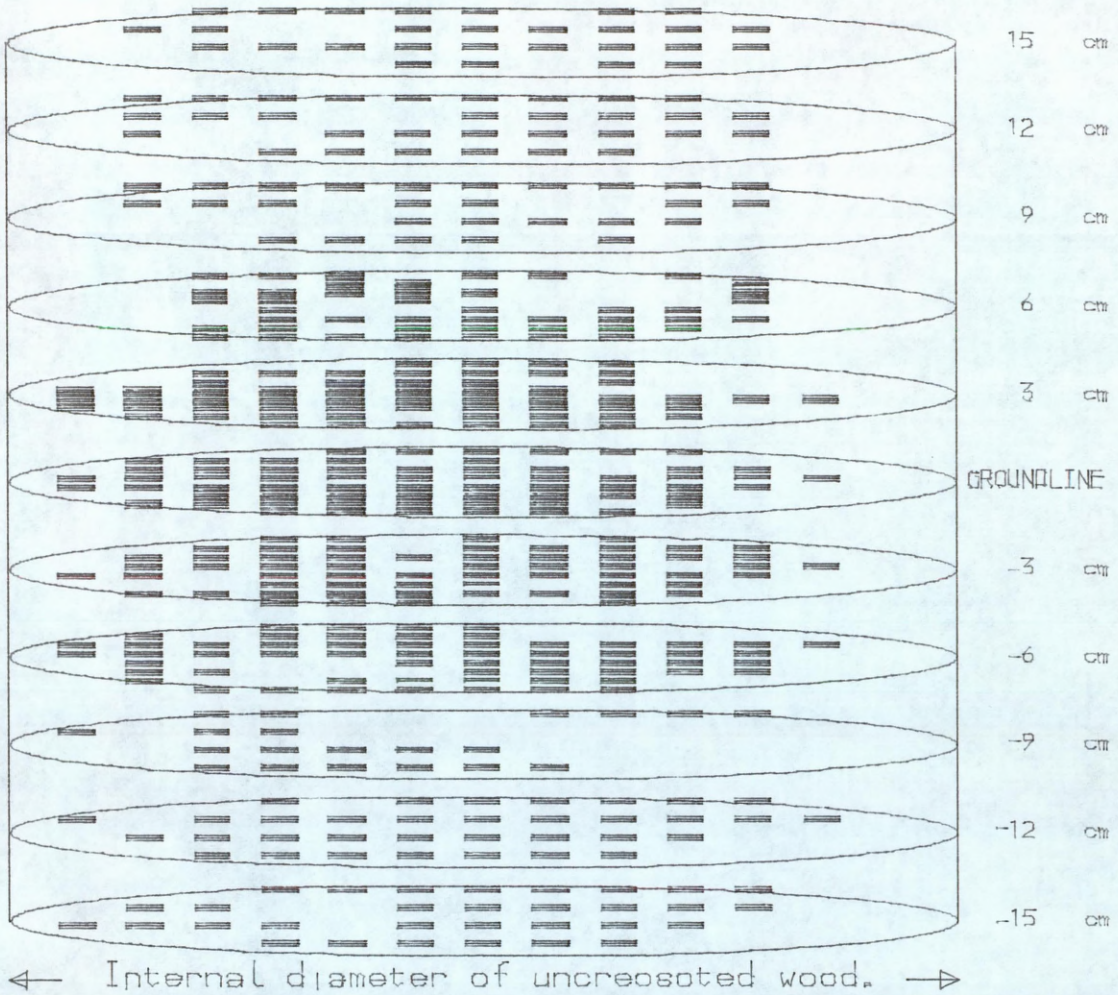
In the following diagrams the pole sections are positioned as in Figure 7.1(1A) with the dissection plane passing directly through the groundline inoculation point. Sample levels at increasing heights above and below the groundline represented here as 3 cms → 15 cms and -3 cms to - 15 cms correspond respectively to levels $A^+ \rightarrow E^+$ and $A^- \rightarrow E^-$ in Figure 7.1(2).

KEY

GREEN:- Trichoderma isolates.

RED :- Lentinus lepideus

BLACK:- Other resident pole organisms



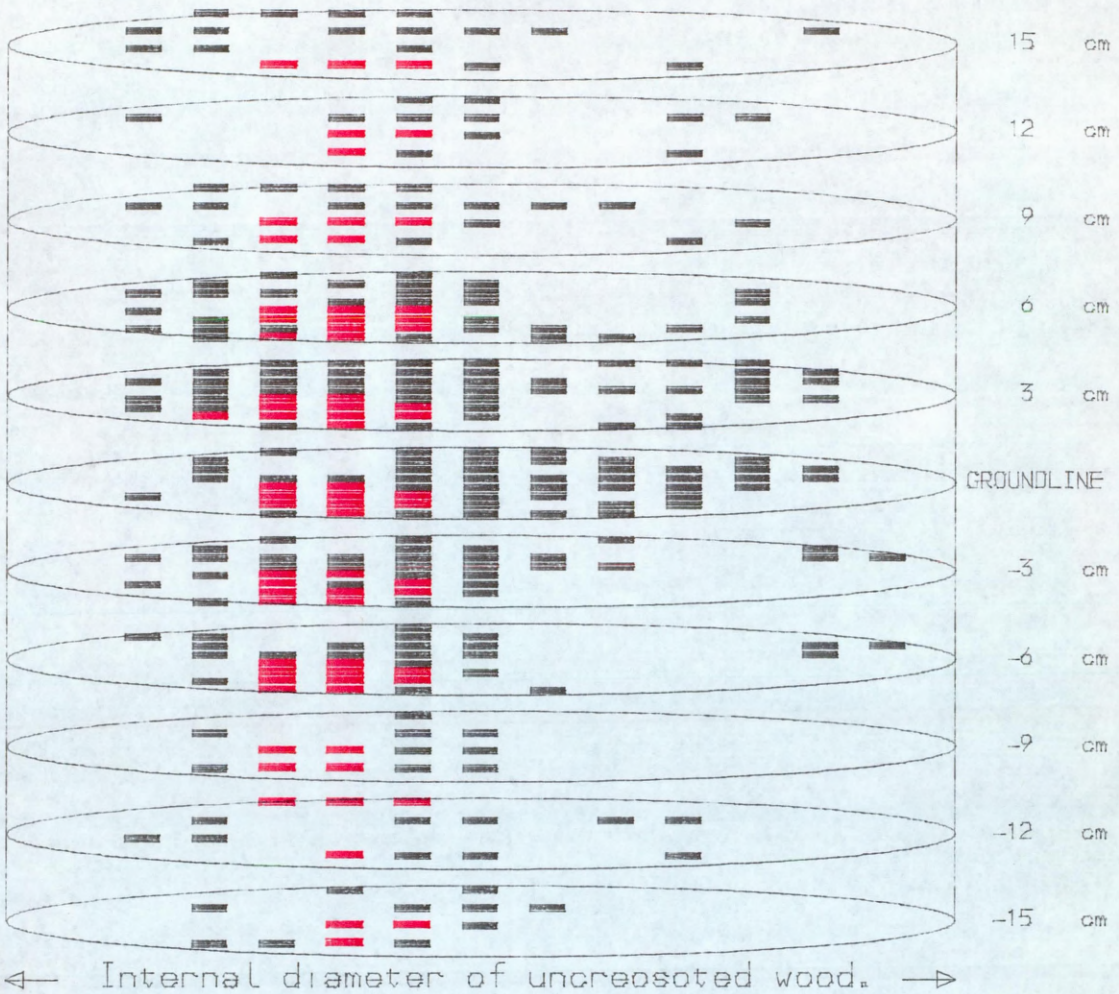
Pole number: XB4 Sectioning date: 9/11/82

FYT pellet inoculation date: none

Lentinus inoculation date: 15/6/81

KEY

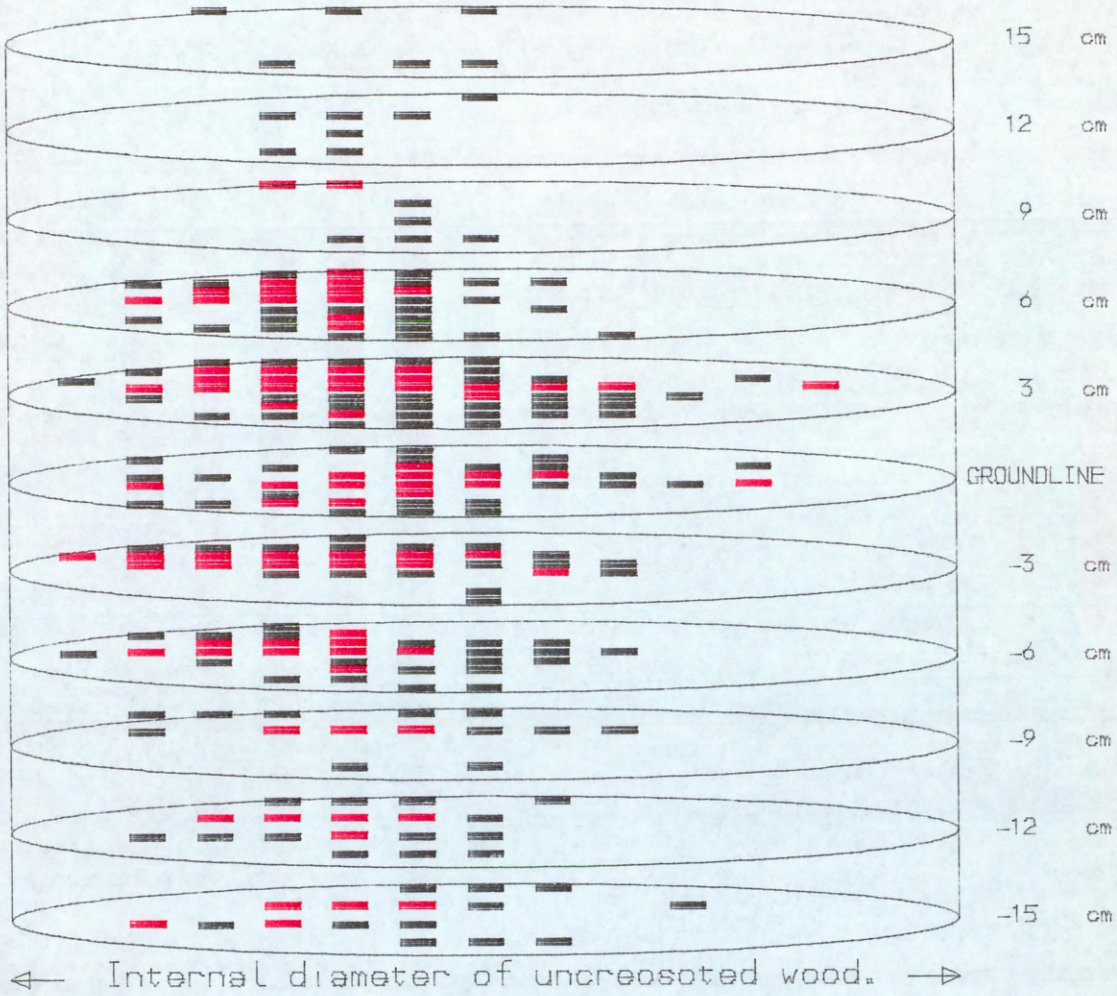
GREEN - *Trichoderma* isolates.
RED - *Lentinus lepideus*
BLACK - Other resident pole organisms



Pole number: XB1B Sectioning date: 14/4/82
FYT pellet inoculation date: none
Lentinus inoculation date: 15/6/81

KEY

GREEN: - Trichoderma isolates.
RED : - Lentinus lepideus
BLACK: - Other resident pole organisms



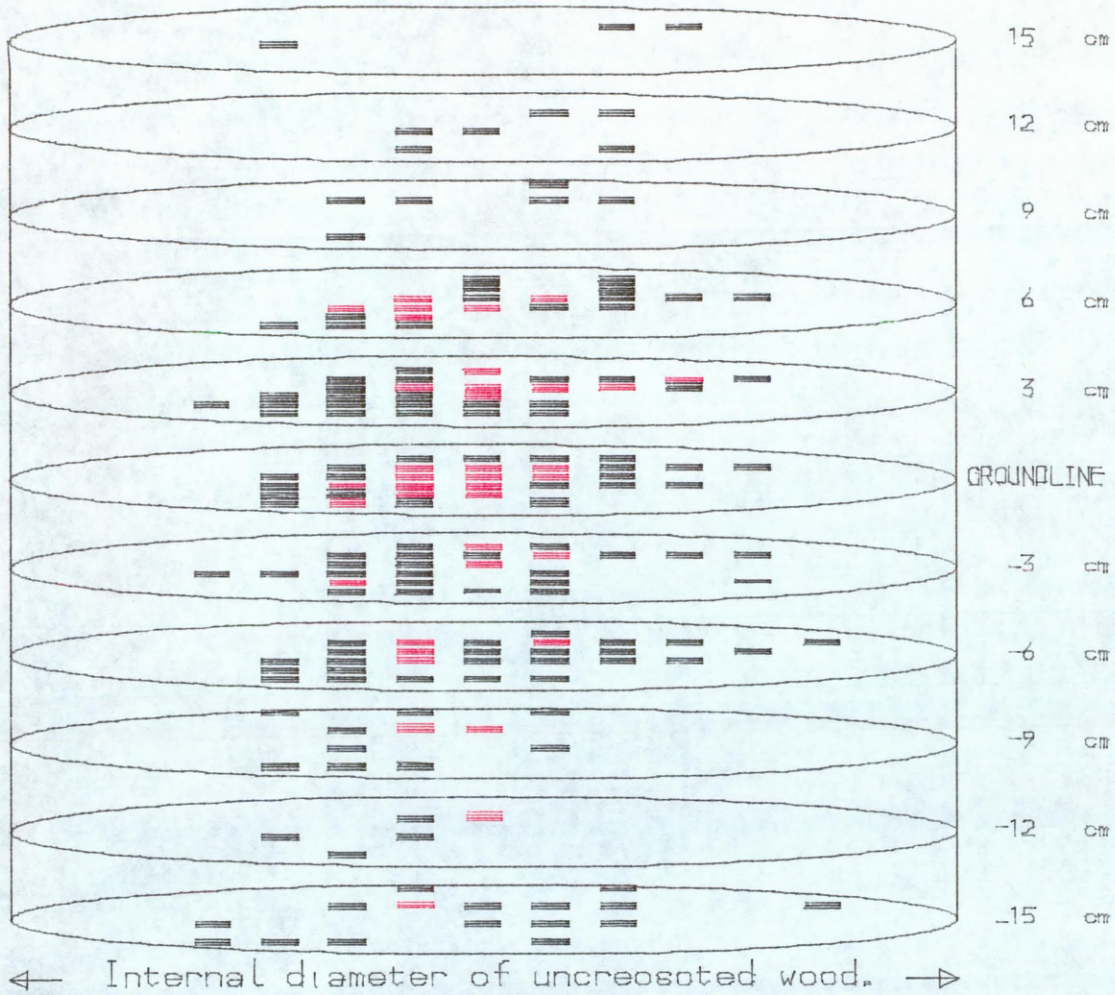
Pole number: XB1F Sectioning date: 1/11/82
FYT pellet inoculation date: none
Lentinus inoculation date: 2/12/81

KEY

GREEN:- Trichoderma isolates.

RED :- Lentinus lepideus

BLACK:- Other resident pole organisms



Pole number: C4F Sectioning date: 23/11/82

FYT pellet inoculation date: none

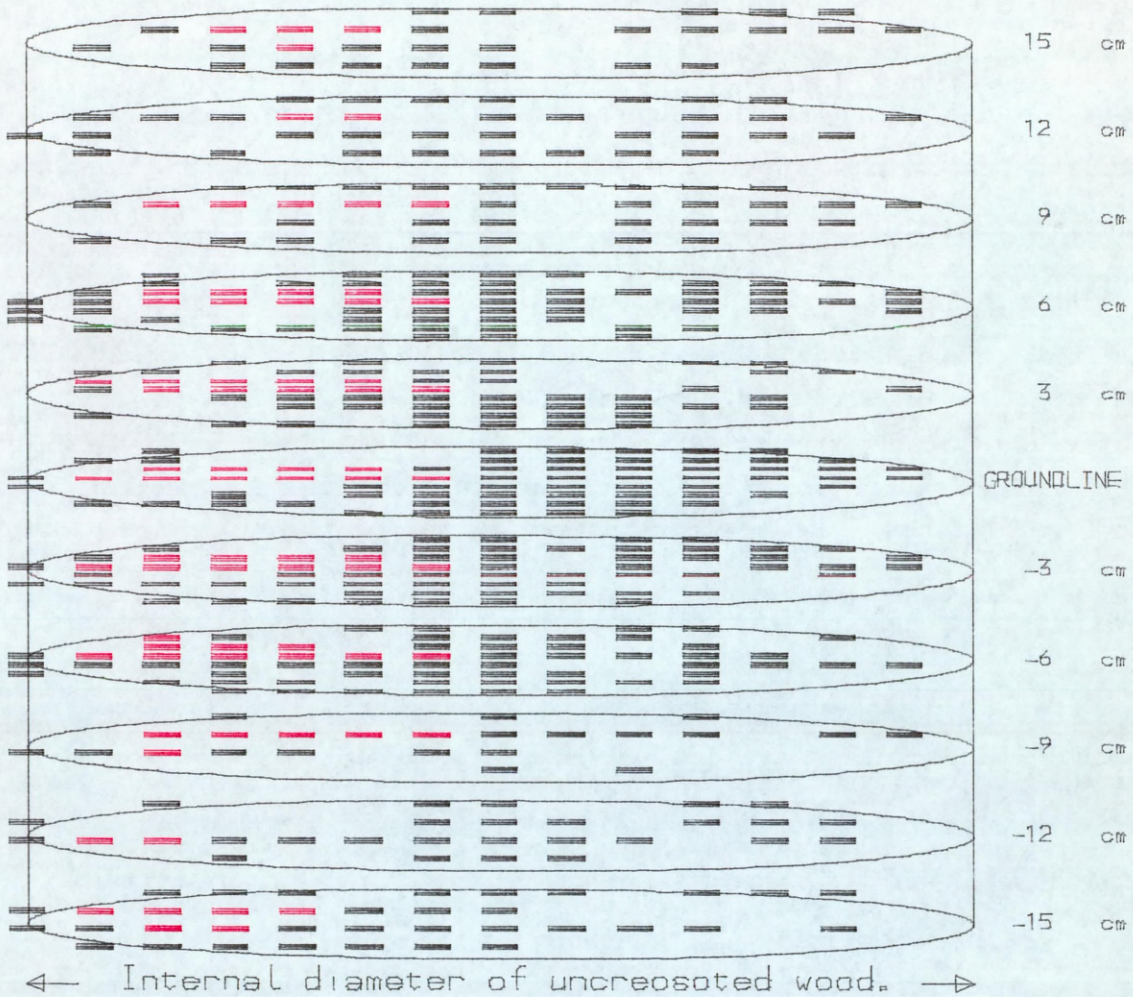
Lentinus inoculation date: 15/6/81

KEY

GREEN:- Trichoderma isolates.

RED :- Lentinus lepideus

BLACK:- Other resident pole organisms



Pole number: B1B Sectioning date: 23/4/83

FYT pellet inoculation date: none

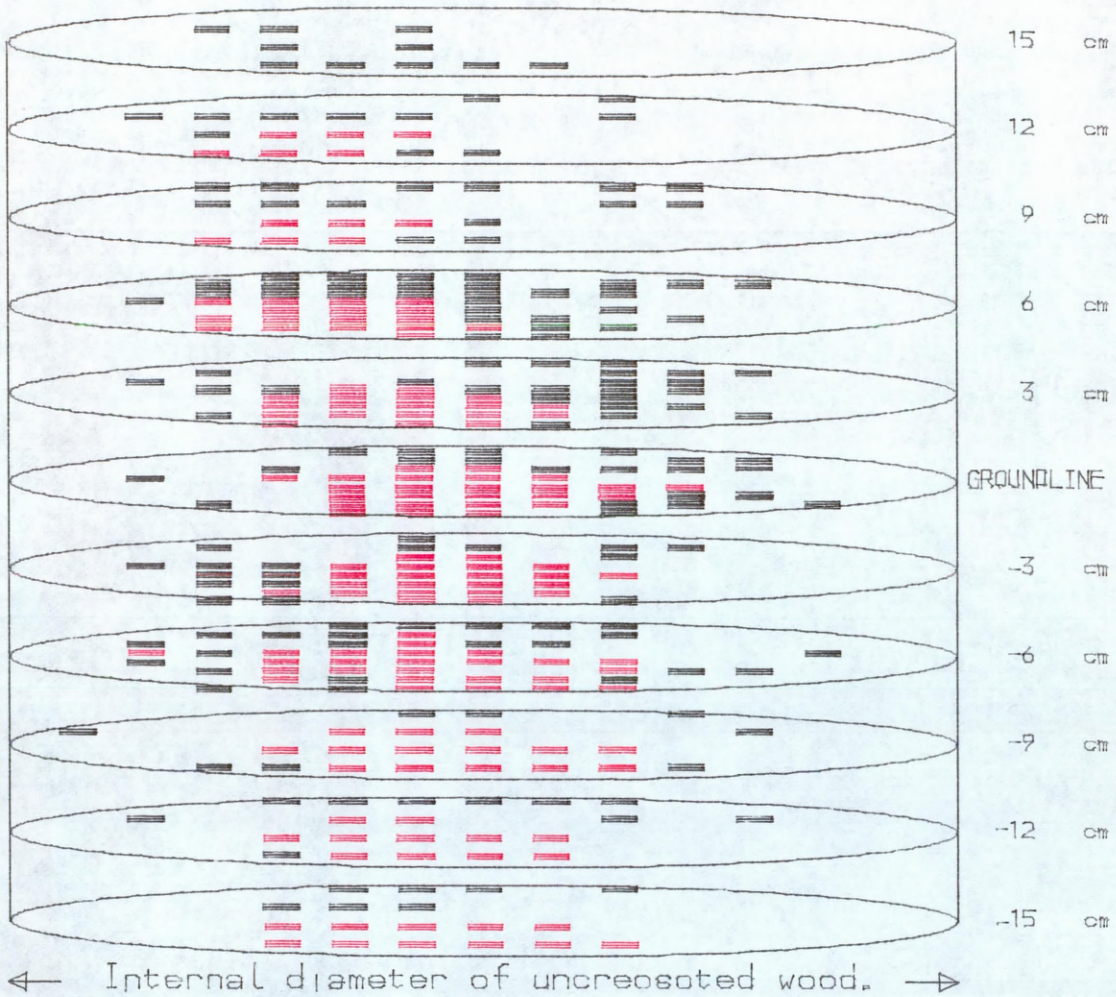
Lentinus inoculation date: 15/6/81

KEY

GREEN:- *Trichoderma* isolates.

RED :- *Lentinus lepideus*

BLACK:- Other resident pole organisms



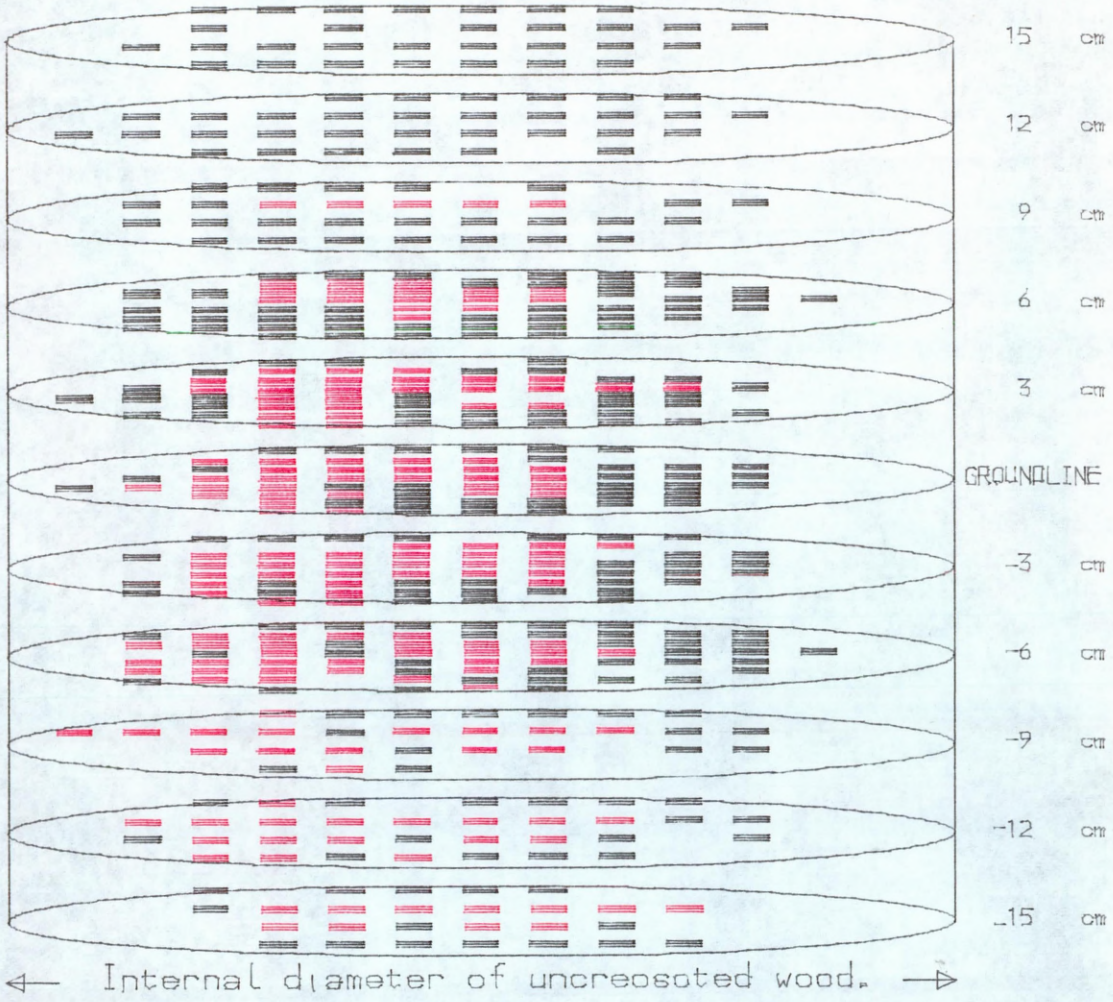
Pole number: XG2 Sectioning date: 30/11/82

FYT pellet inoculation date: none

Lentinus inoculation date: 15/6/81

KEY

GREEN: - Trichoderma isolates.
RED : - Lentinus lepideus
BLACK: - Other resident pole organisms



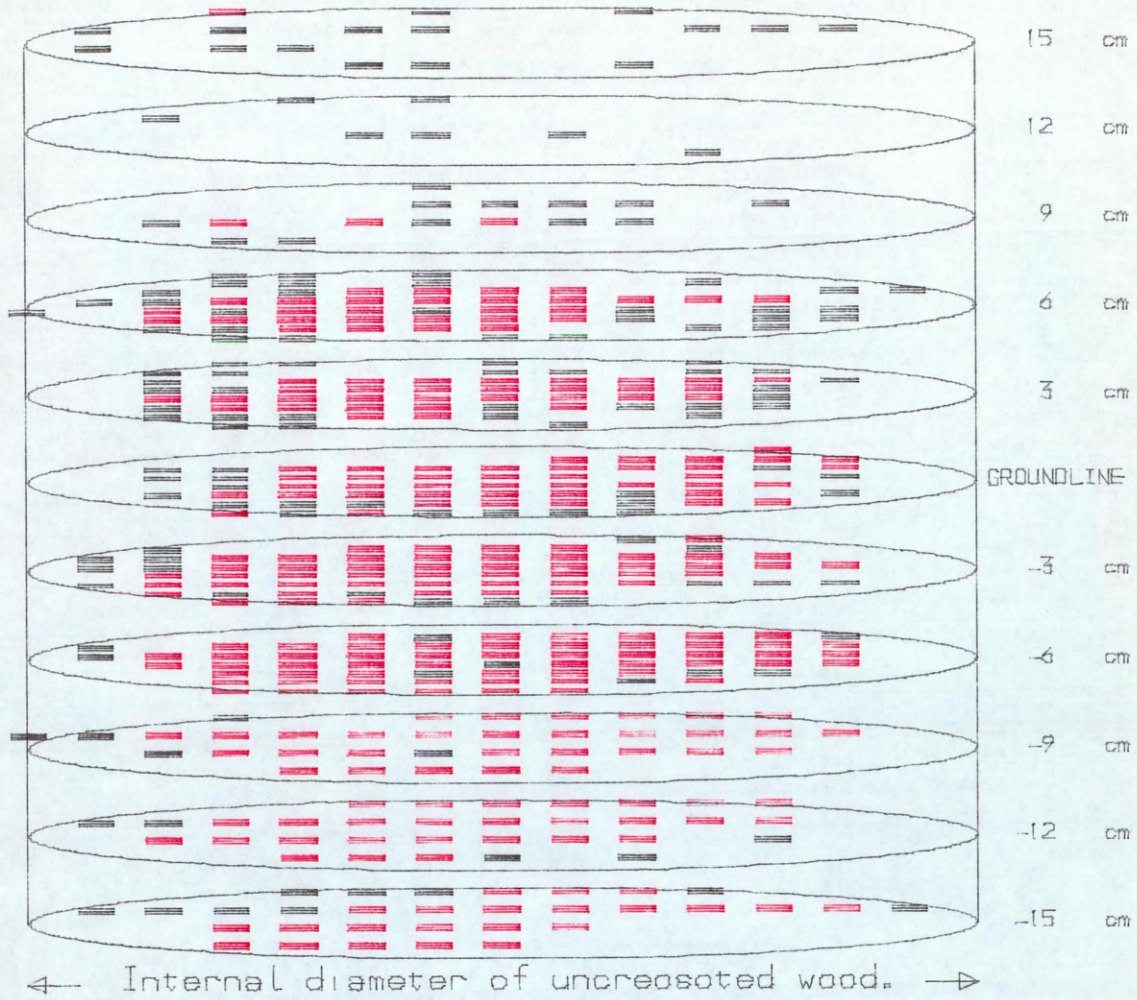
Pole number: C4B Sectioning date: 8/12/82
FYT pellet inoculation date: none
Lentinus inoculation date: 2/12/81

KEY

GREEN:- *Trichoderma* isolates.

RED :- *Lentinus lepideus*

BLACK:- Other resident pole organisms



Pole number: H2 Sectioning date: 14/6/83

FYT pellet inoculation date: none

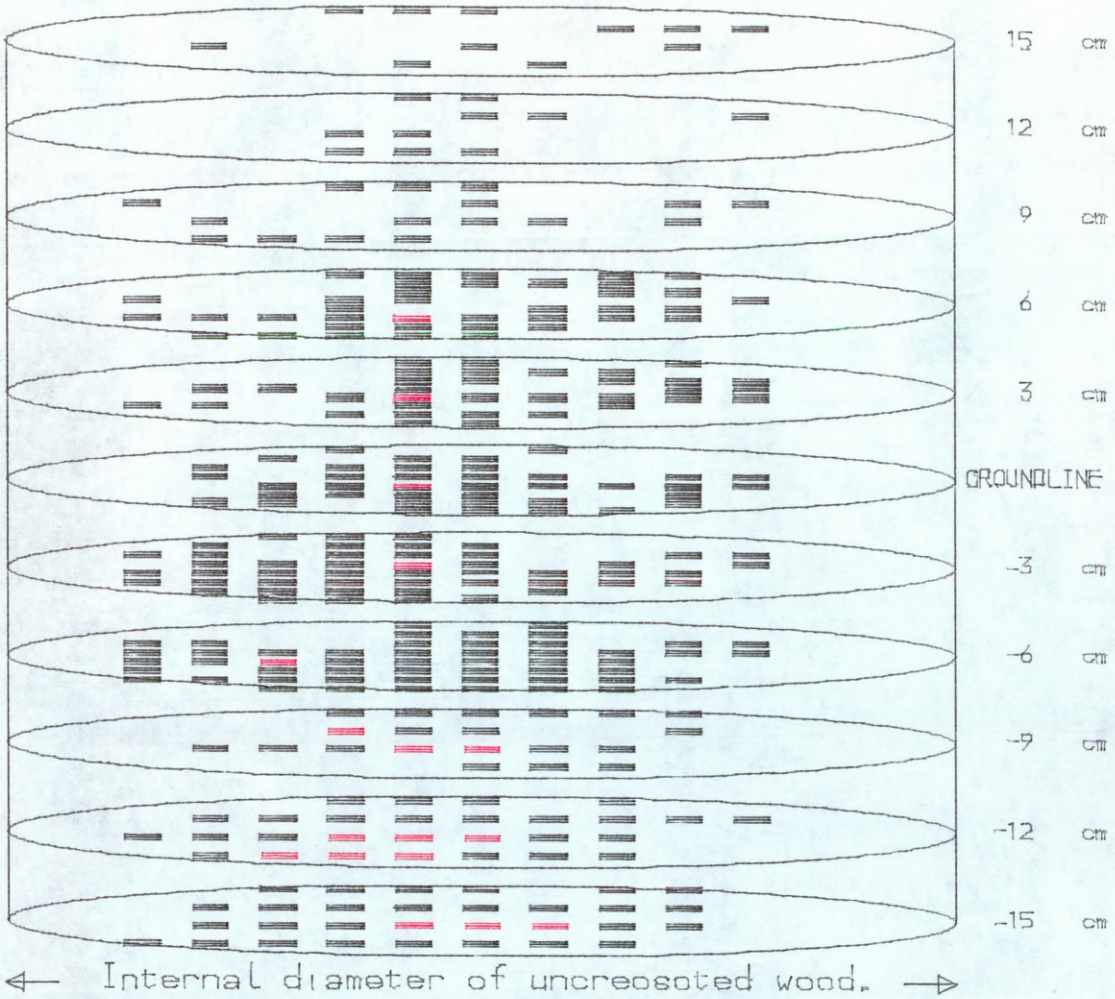
Lentinus inoculation date: 15/6/81

KEY

GREEN:- Trichoderma isolates.

RED :- Lentinus lepideus

BLACK:- Other resident pole organisms



Pole number: XB2 Sectioning date: 23/11/82

FYT pellet inoculation date: none

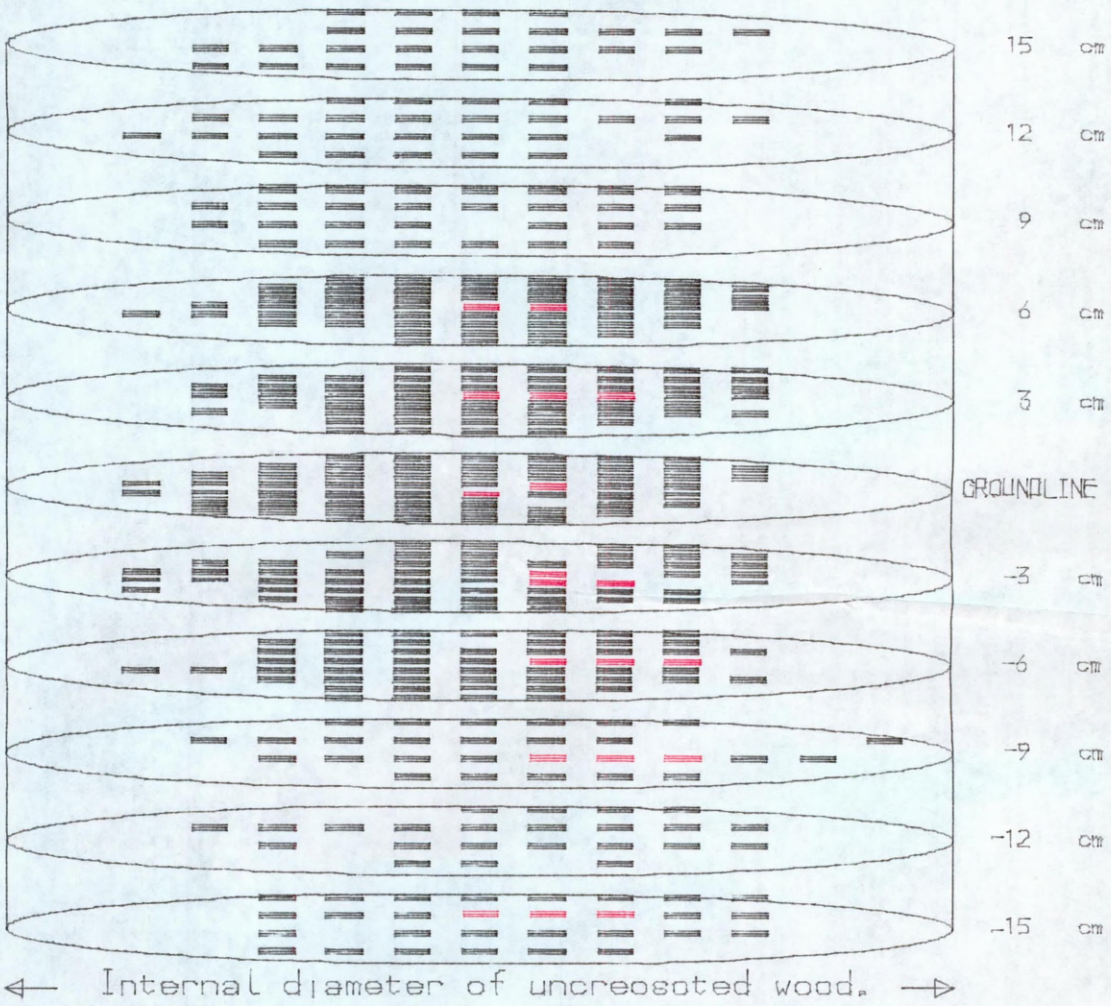
Lentinus inoculation date: 15/6/81

KEY

GREEN:- Trichoderma isolates.

RED :- Lentinus lepideus

BLACK:- Other resident pole organisms



Pole number: XG3 Sectioning date: 16/11/82

FYT pellet inoculation date: none

Lentinus inoculation date: 2/12/81

APPENDIX III

Diagrams showing the distribution of *Trichoderma*, *L. lepideus* and pole resident organisms isolated from sections removed from the groundlines of 10 poles inoculated with Binab FYT pellets in June 1981 and then subsequently inoculated with *L. lepideus* in December 1981.

(All poles were inoculated with both organisms at the groundline and the diagrams represent the distribution as seen from an angle 10° above the plane on which the section sits.)

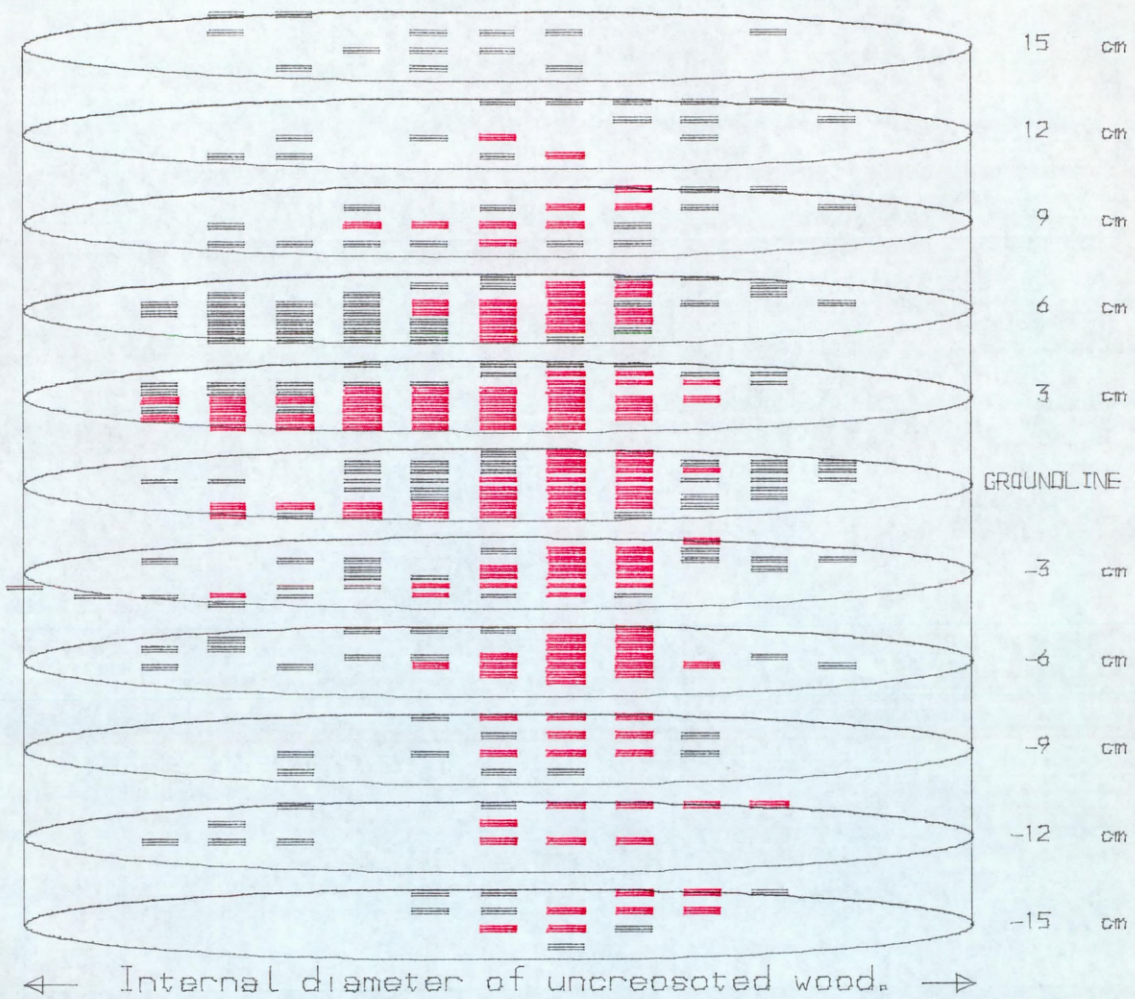
In the following diagrams the pole sections are positioned as in Figure 7.1(1A) with the dissection plane passing directly through the groundline inoculation point. Sample levels at increasing heights above and below the groundline represented here as 3 cms → 15 cms and -3 cms → -15 cms correspond respectively to levels $A^+ \rightarrow E^+$ and $A^- \rightarrow E^-$ in Figure 7.1(2).

KEY

GREEN:- Trichoderma isolates.

RED :- Lentinus lepideus

BLACK:- Other resident pole organisms



Pole number: I3 Sectioning date: 18/4/83

FYT pellet inoculation date: 18/6/81

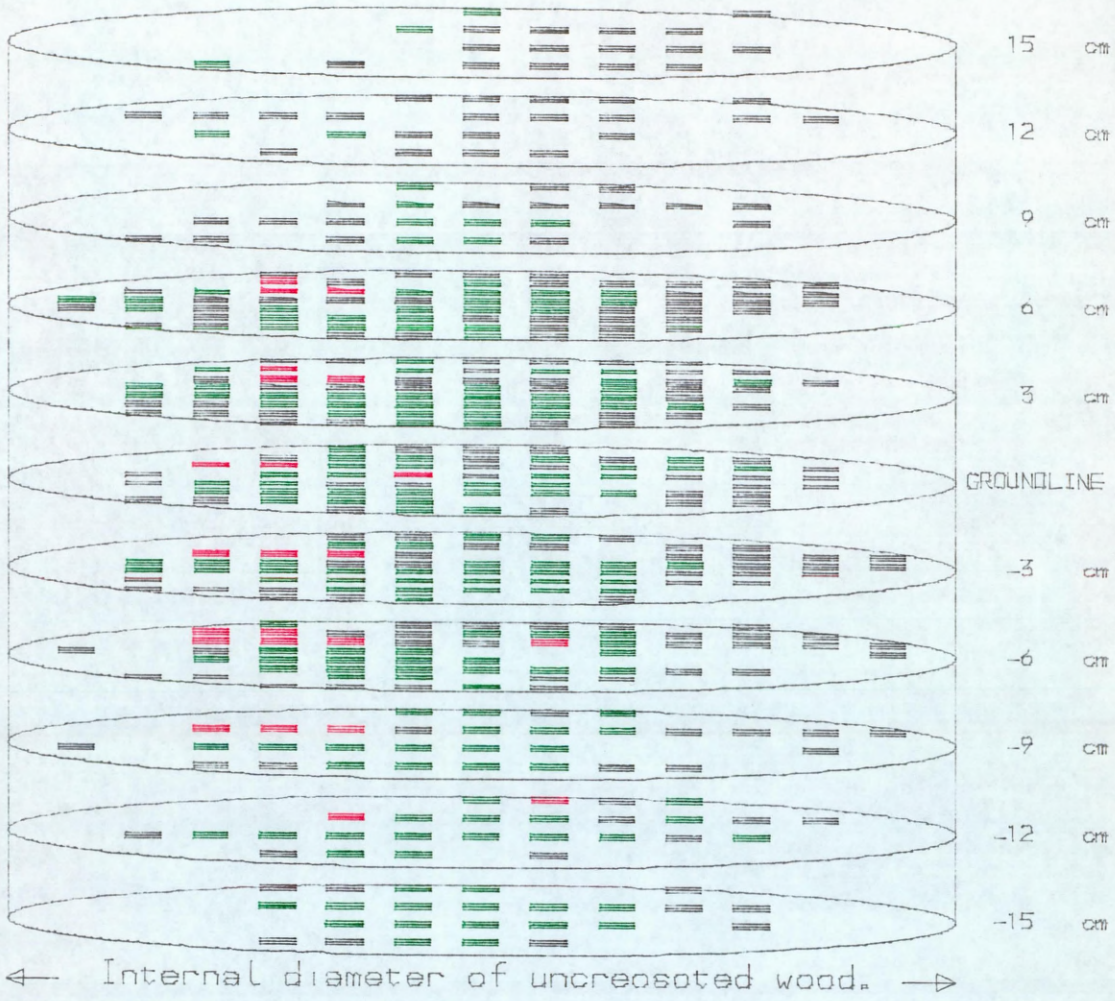
Lentinus inoculation date: 2/12/81

KEY

GREEN:- *Trichoderma* isolates.

RED :- *Lentinus lepideus*

BLACK:- Other resident pole organisms



Pole number: J4 Sectioning date: 4/5/83

FYT pellet inoculation date: 18/6/81

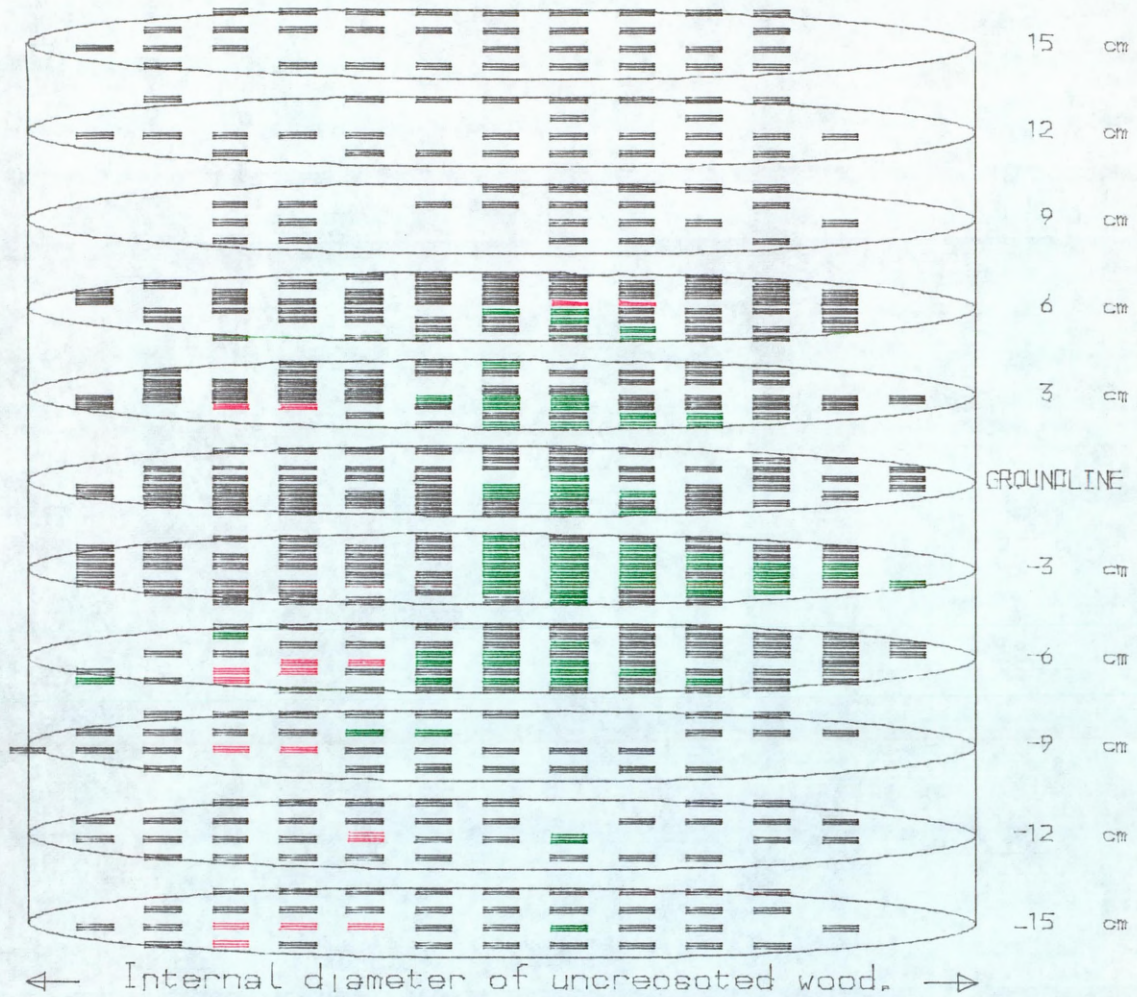
Lentinus inoculation date: 2/12/81

KEY

GREEN:- *Trichoderma* isolates.

RED :- *Lentinus lepideus*

BLACK:- Other resident pole organisms



Pole number: K2L Sectioning date: 9/1/83

FYT pellet inoculation date: 18/6/81

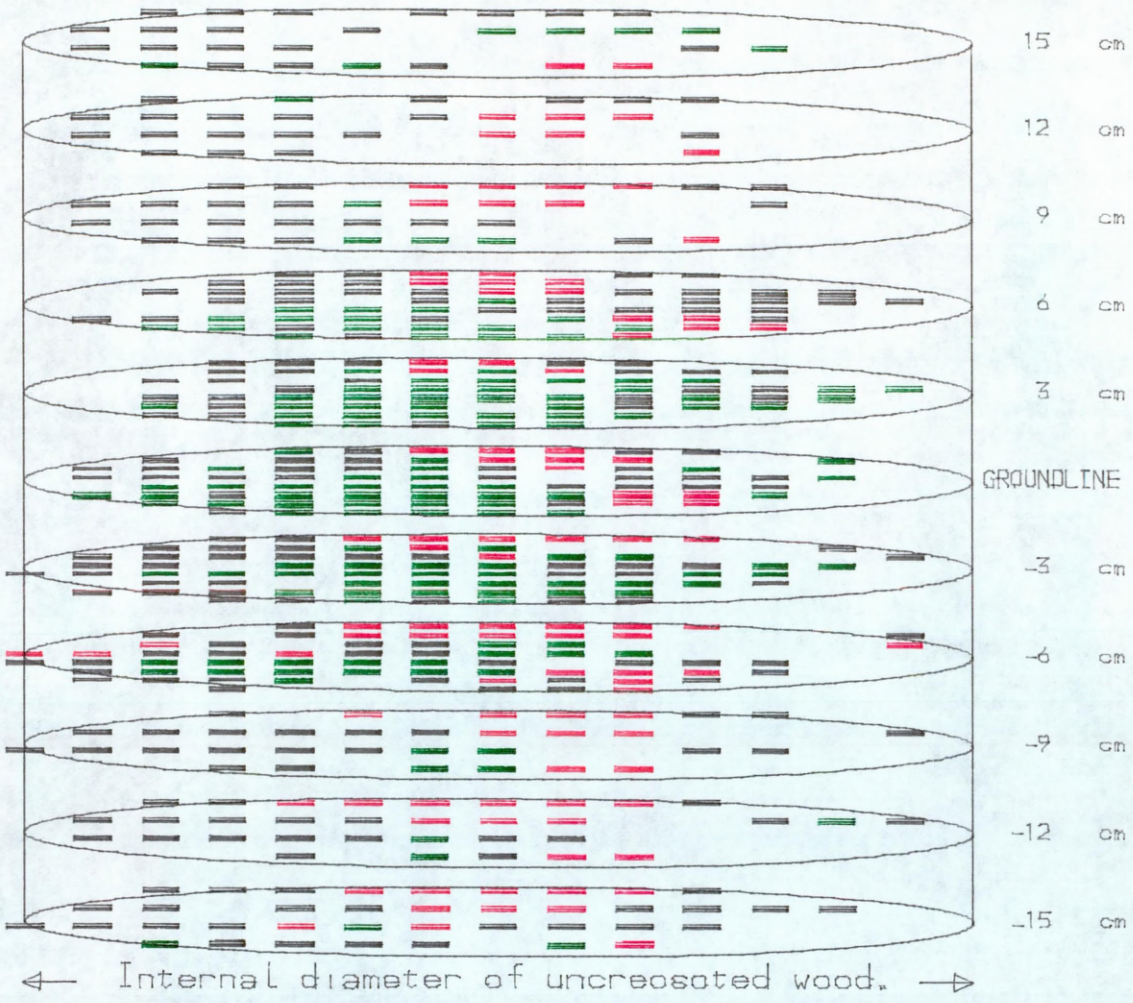
Lentinus inoculation date: 2/12/81

KEY

GREEN:- *Trichoderma* isolates.

RED :- *Lentinus lepideus*

BLACK:- Other resident pole organisms



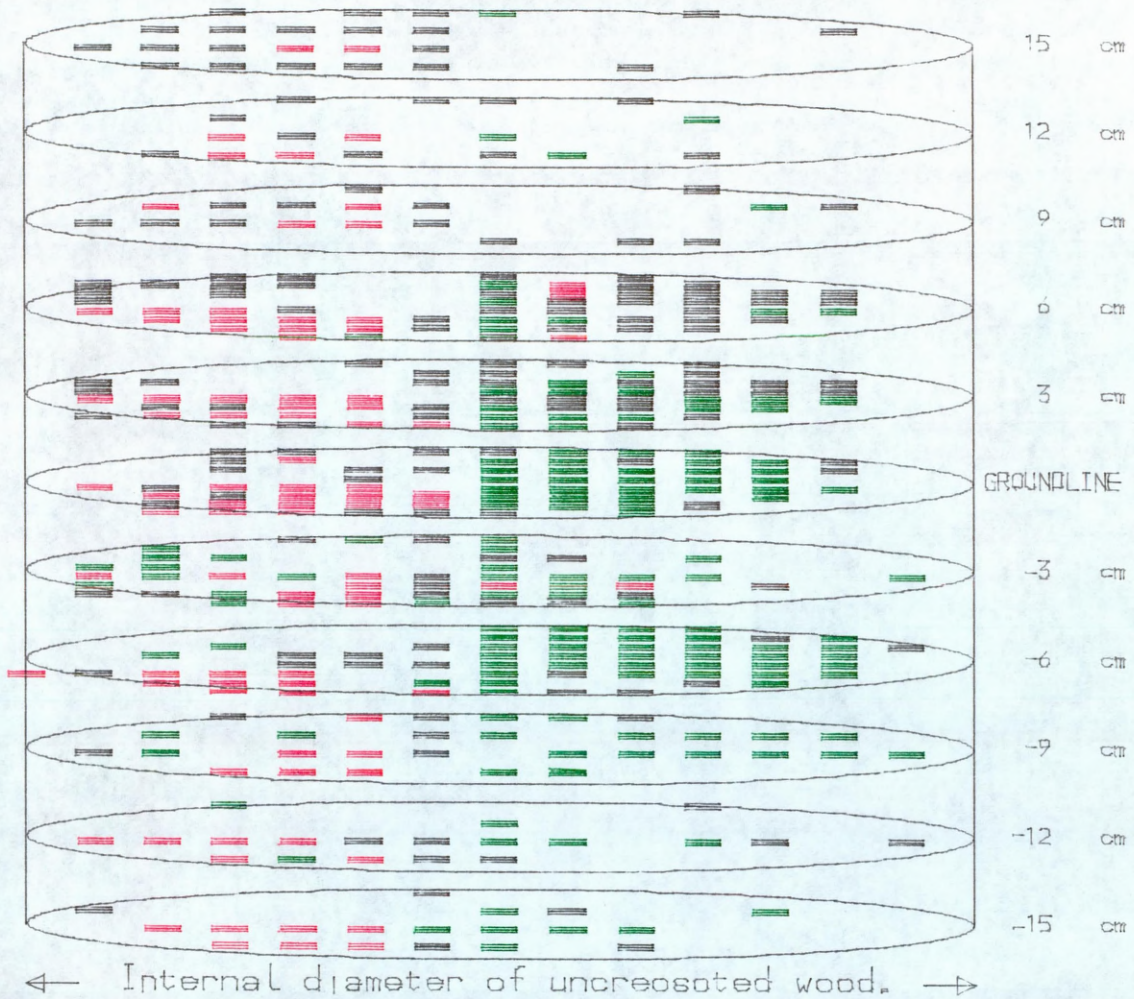
Pole number: J3 Sectioning date: 15/12/82

FYT pellet inoculation date: 18/6/81

Lentinus inoculation date: 2/12/81

KEY

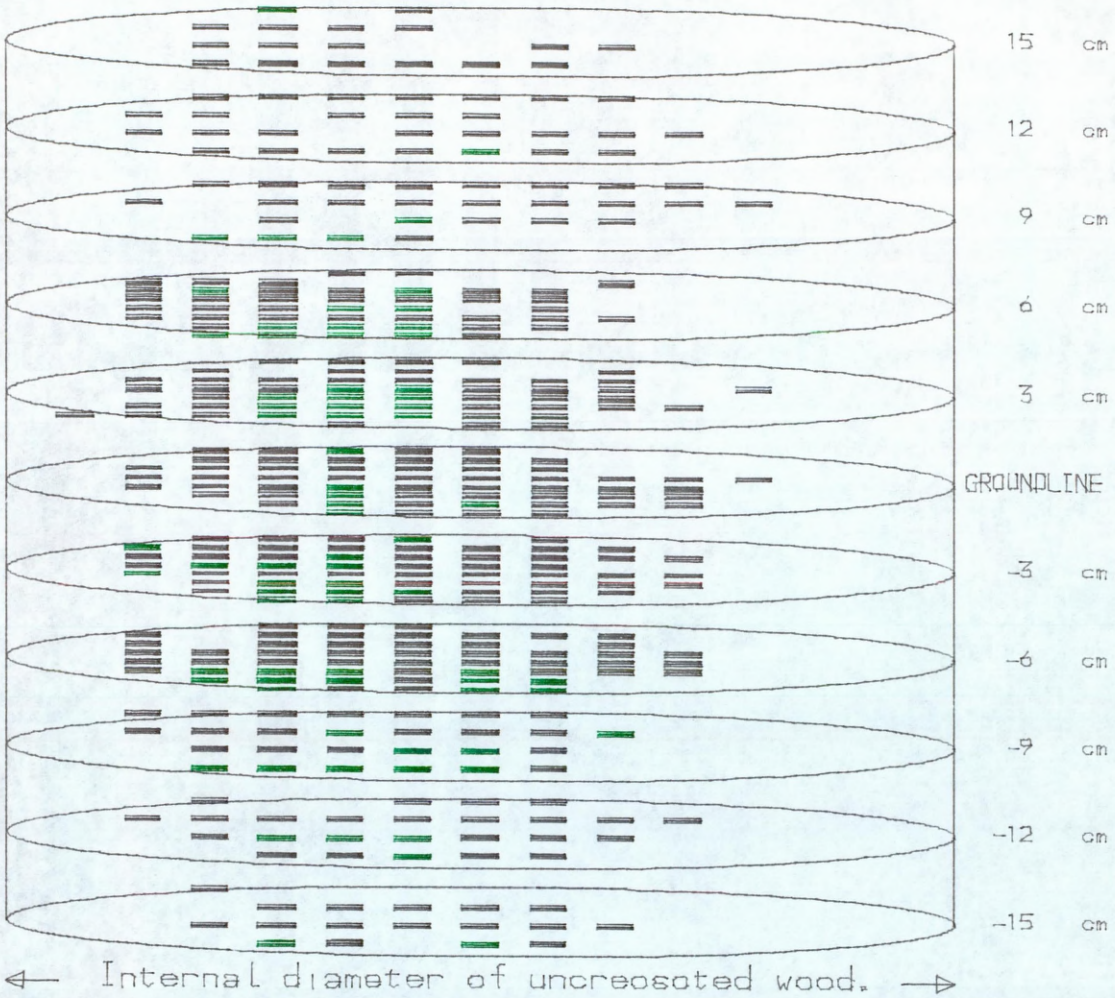
GREEN:- Trichoderma isolates.
RED :- Lentinus lepideus
BLACK:- Other resident pole organisms



Pole number: K2R Sectioning date: 10/1/83
FYT pellet inoculation date: 18/6/81
Lentinus inoculation date: 2/12/81

KEY

GREEN:- Trichoderma isolates.
RED :- Lentinus lepideus
BLACK:- Other resident pole organisms



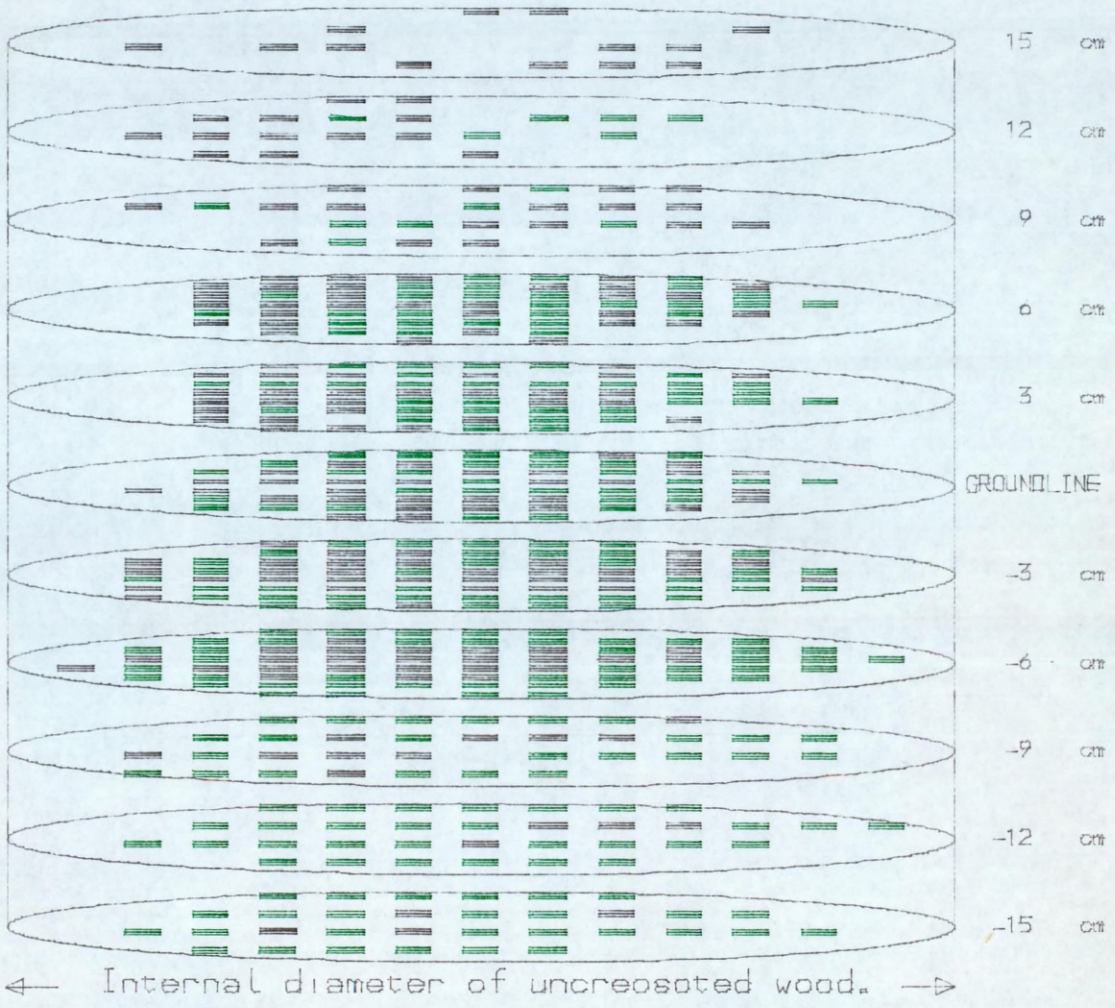
Pole number: J2 Sectioning date: 13/12/82
FYT pellet inoculation date: 18/6/81
Lentinus inoculation date: 2/12/81

KEY

GREEN: - *Trichoderma* isolates.

RED : - *Lentinus lepideus*

BLACK: - Other resident pole organisms



Pole number: I1 Sectioning date: 6/3/83

FYT pellet inoculation date: 18/6/81

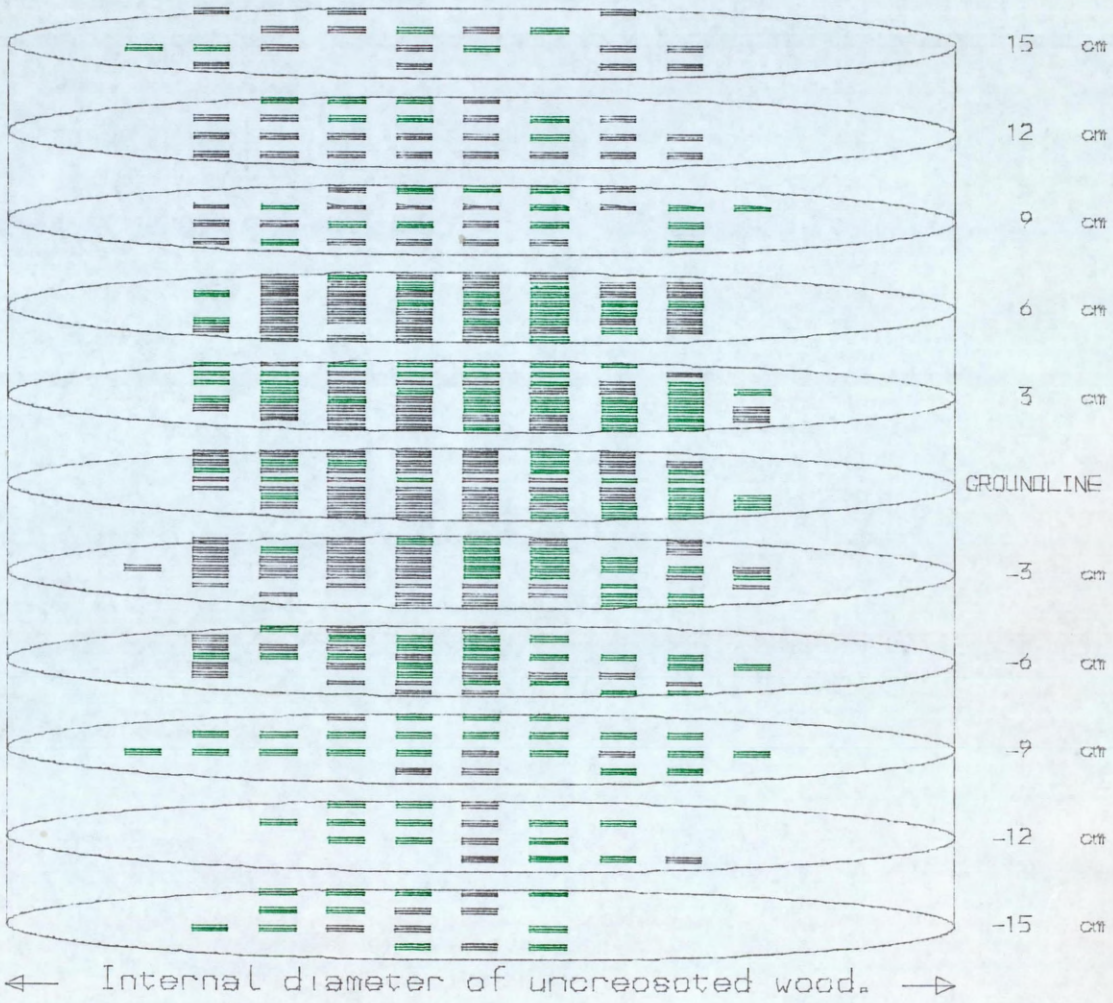
Lentinus inoculation date: 2/12/81

KEY

GREEN:- Trichoderma isolates.

RED :- Lentinus lepideus

BLACK:- Other resident pole organisms



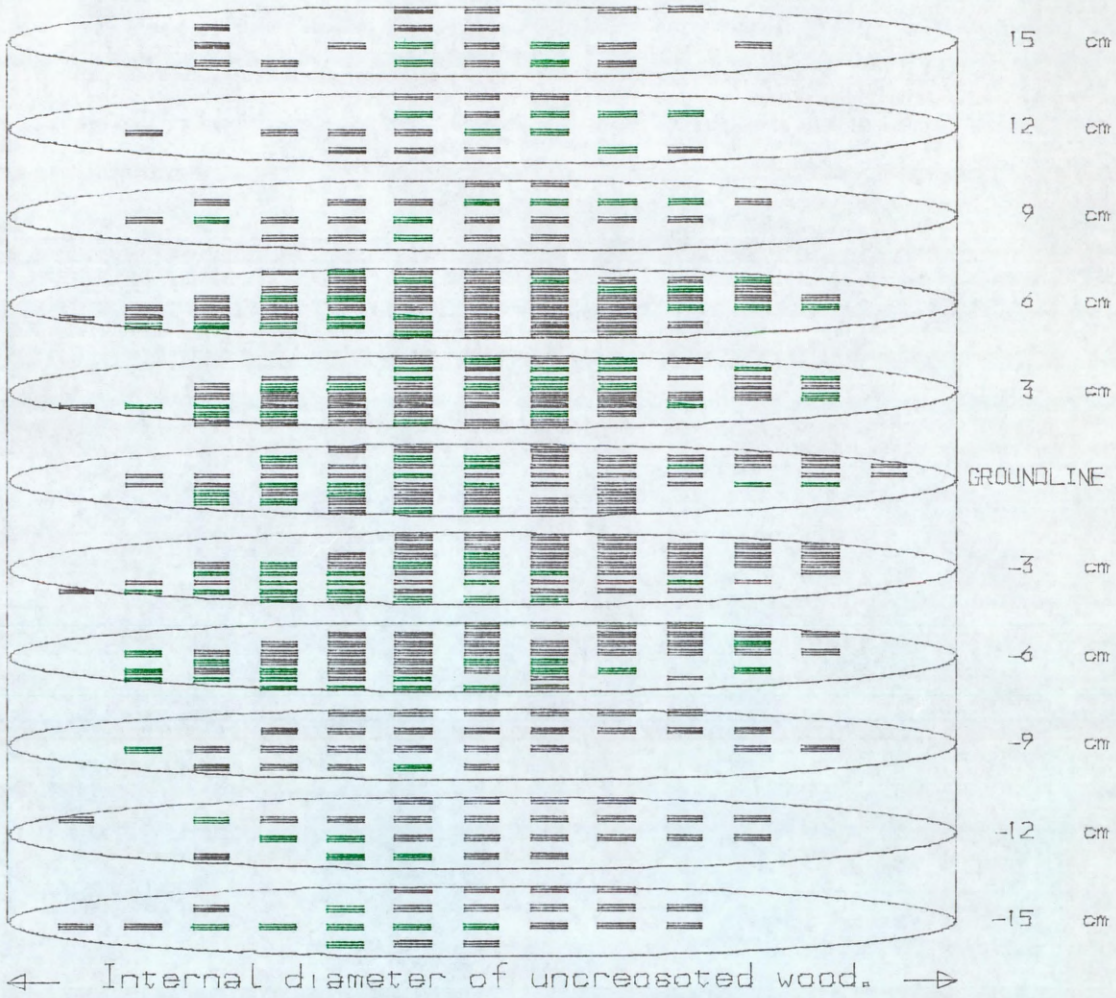
Pole number: I2 Sectioning date: 12/5/83

FYT pellet inoculation date: 18/6/81

Lentinus inoculation date: 2/12/81

KEY

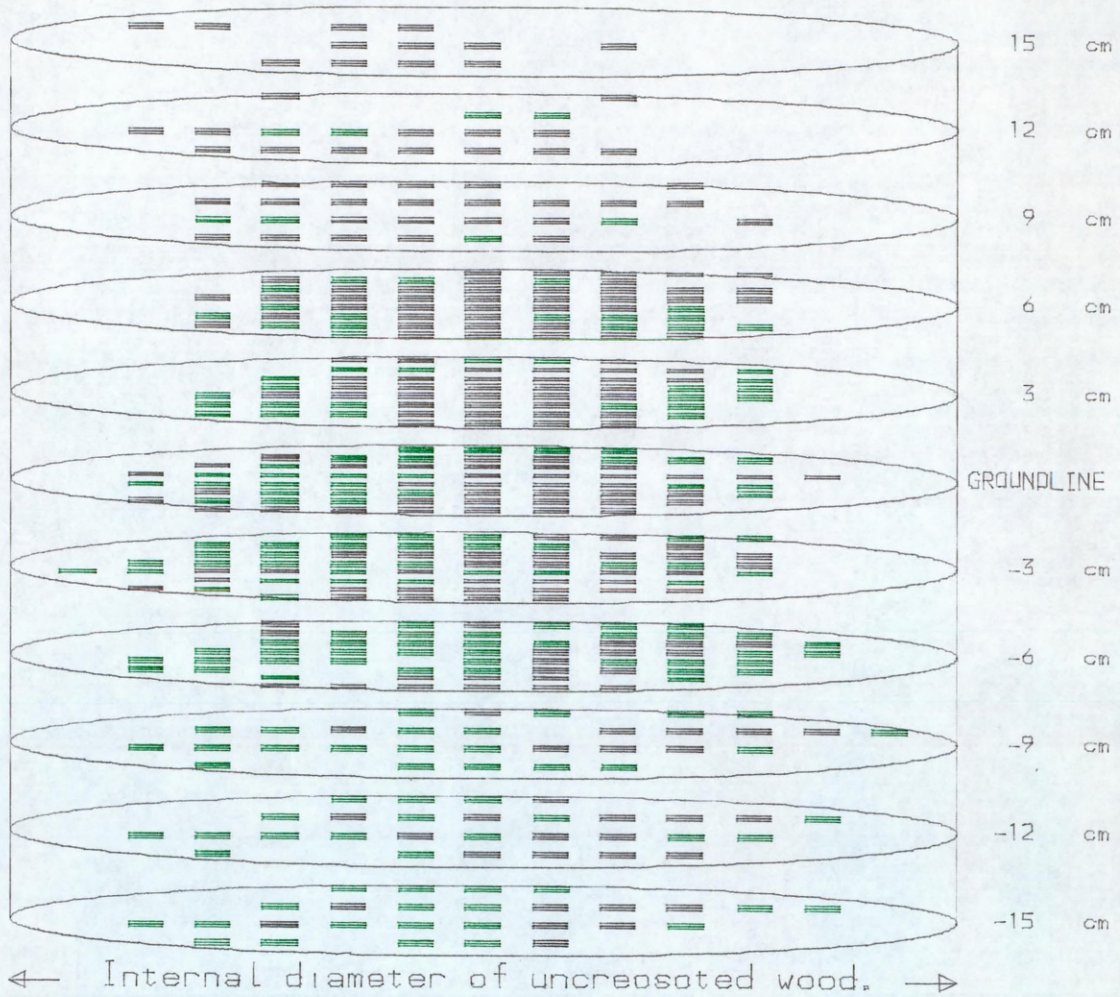
GREEN:- *Trichoderma* isolates.
RED :- *Lentinus lepideus*
BLACK:- Other resident pole organisms



Pole number: I5 Sectioning date: 10/5/83
FYT pellet inoculation date: 18/6/81
Lentinus inoculation date: 2/12/81

KEY

GREEN:- *Trichoderma* isolates.
RED :- *Lentinus lepideus*
BLACK:- Other resident pole organisms



Pole number: J1 Sectioning date: 20/4/83
FYT pellet inoculation date: 18/6/81
Lentinus inoculation date: 2/12/81

APPENDIX IV

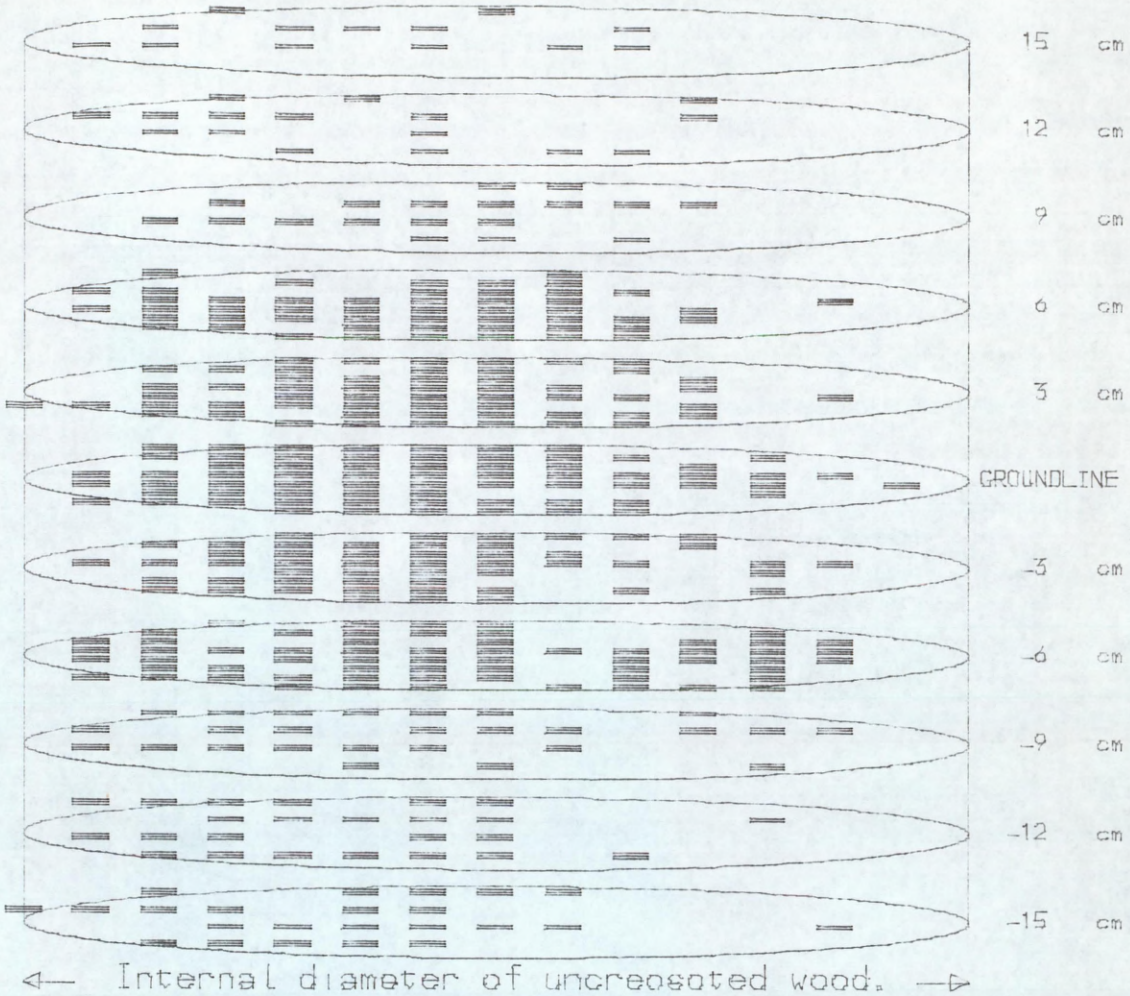
Diagrams showing the distribution of *Trichoderma*, *L. lepideus* and pole resident organisms isolated from sections removed from the groundlines of 10 poles artificially inoculated with *L. lepideus* and subsequently inoculated with Binab FYT pellets.

(All poles were inoculated with both organisms at the groundline and the diagrams represent the distribution as seen from an angle 10° above the plane on which the section sits.)

In the following diagrams the pole sections are positioned as in Figure 7.1(1A) with the dissection plane passing directly through the groundline inoculation point. Sample levels at increasing heights above and below the groundline represented here as 3 cms → 15 cms and -3 cms → -15 cms correspond respectively to levels $A^+ \rightarrow E^+$ and $A^- \rightarrow E^-$ in Figure 7.1(2).

KEY

GREEN:- Trichoderma isolates.
RED :- Lentinus lepideus
BLACK:- Other resident pole organisms



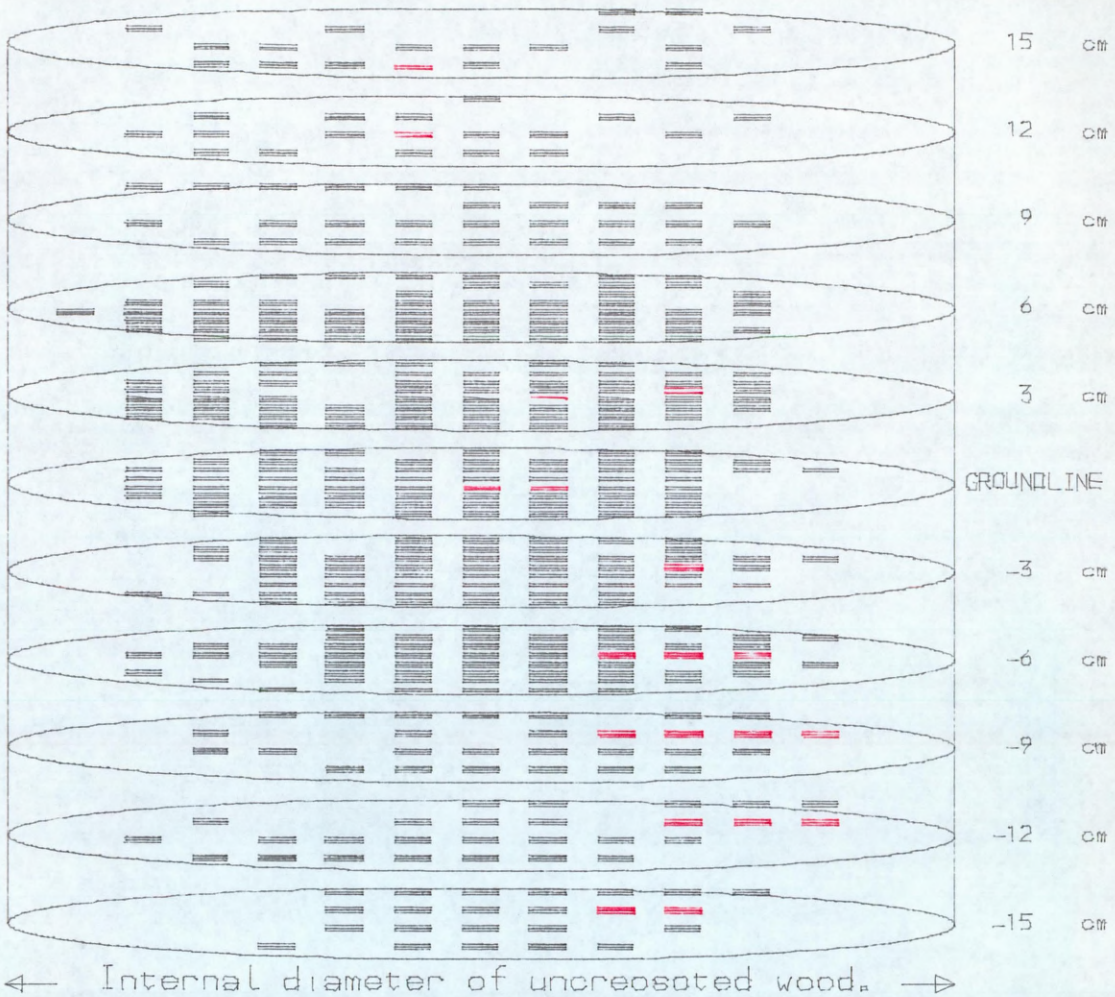
Pole number: XF4 Sectioning date: 22/6/83
FYT pellet inoculation date: 11/5/82
Lentinus inoculation date: 2/12/81

KEY

GREEN:- Trichoderma isolates.

RED :- Lentinus lepideus

BLACK:- Other resident pole organisms



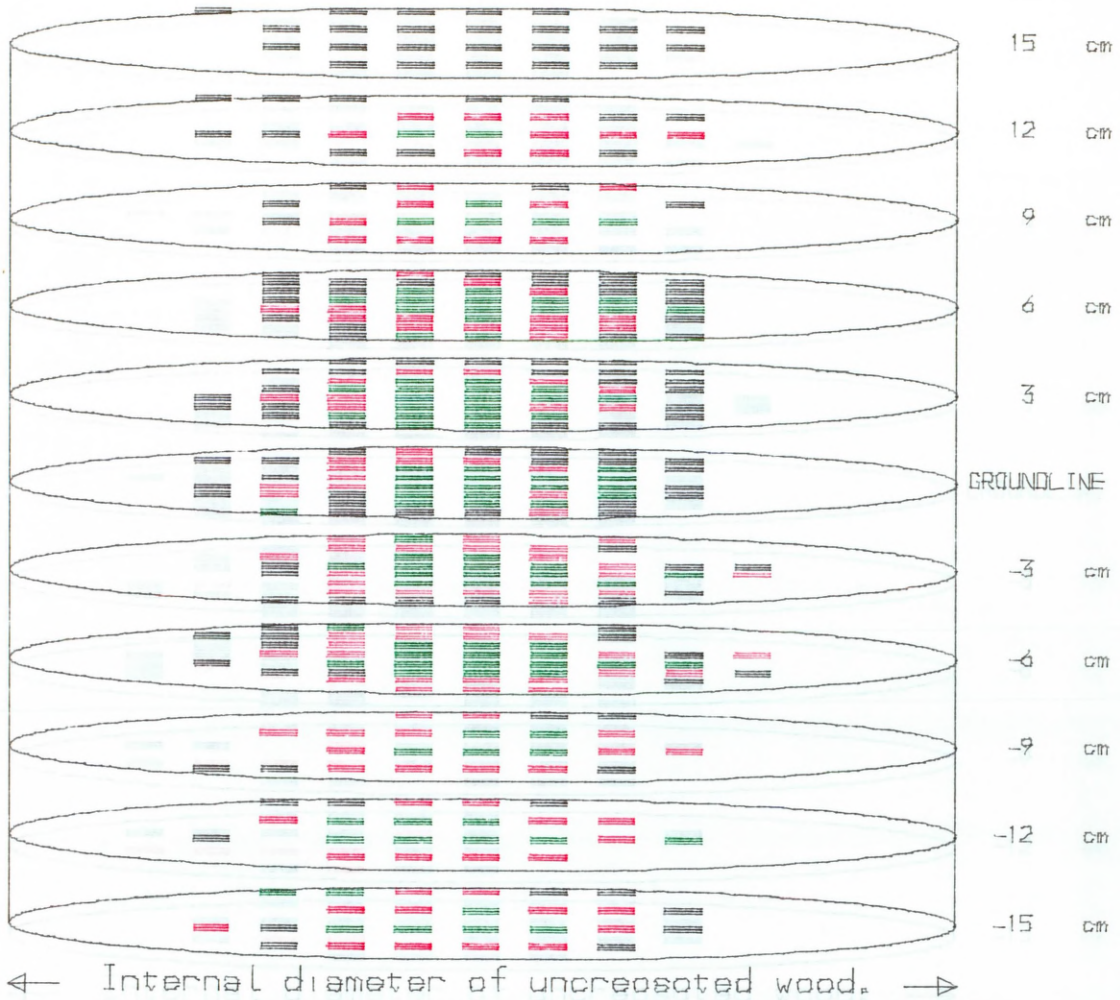
Pole number: XD2 Sectioning date: 24/6/83

FYT pellet inoculation date: 11/5/82

Lentinus inoculation date: 2/12/81

KEY

GREEN:- Trichoderma isolates.
RED :- Lentinus lepideus
BLACK:- Other resident pole organisms



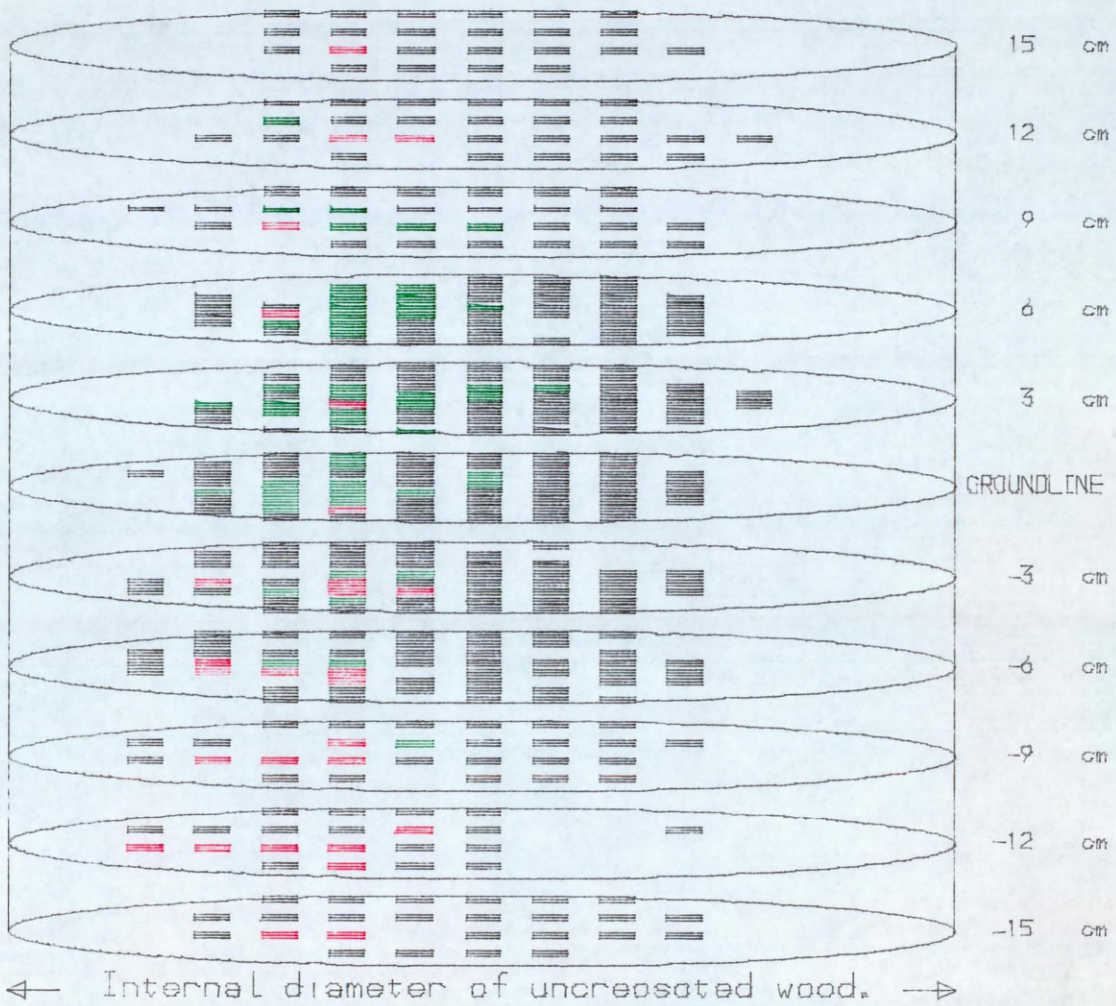
Pole number: XD3 Sectioning date: 27/6/83
FYT pellet inoculation date: 11/5/82
Lentinus inoculation date: 2/12/81

KEY

GREEN: - *Trichoderma* isolates.

RED : - *Lentinus lepideus*

BLACK: - Other resident pole organisms



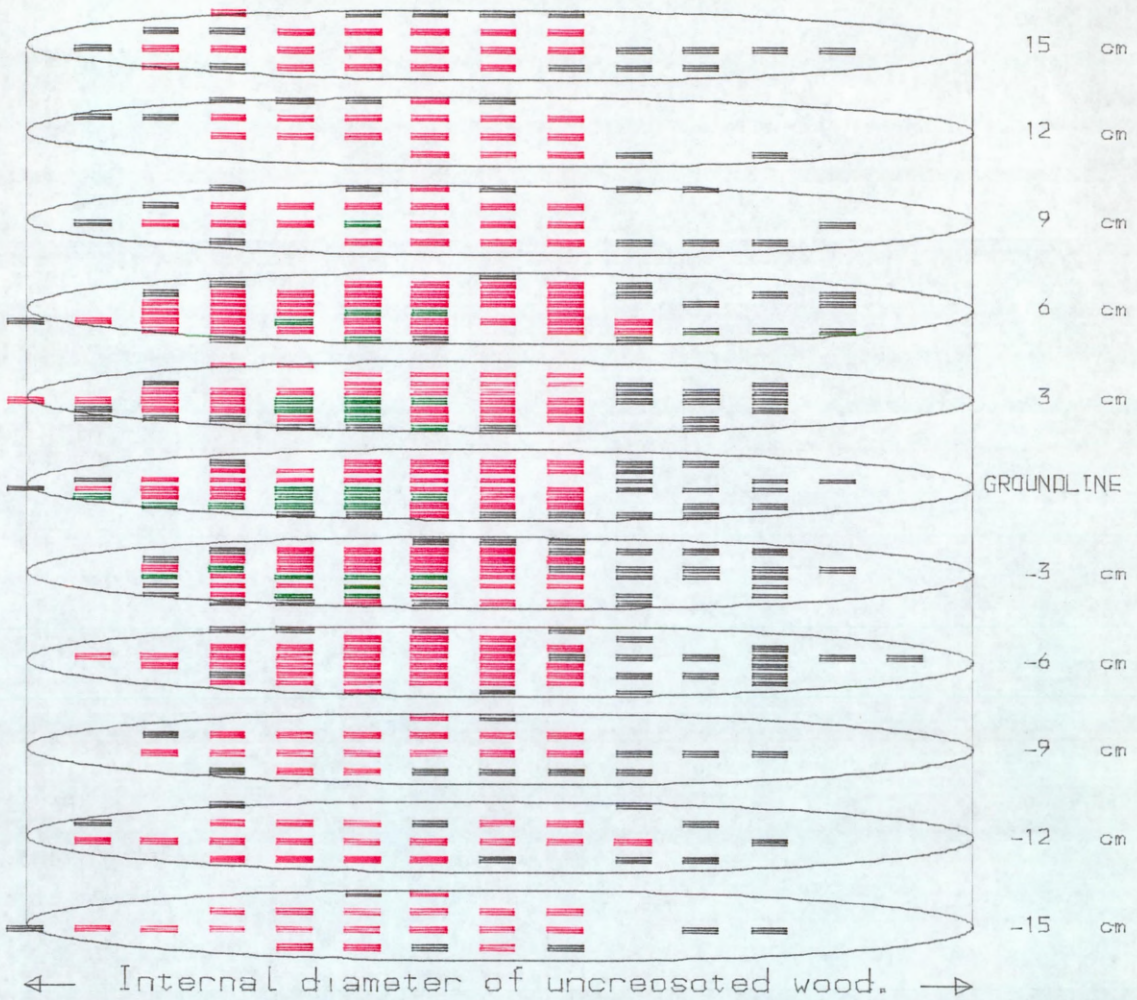
Pole number: XF1 Sectioning date: 23/6/83

FYT pellet inoculation date: 11/5/82

Lentinus inoculation date: 2/12/81

KEY

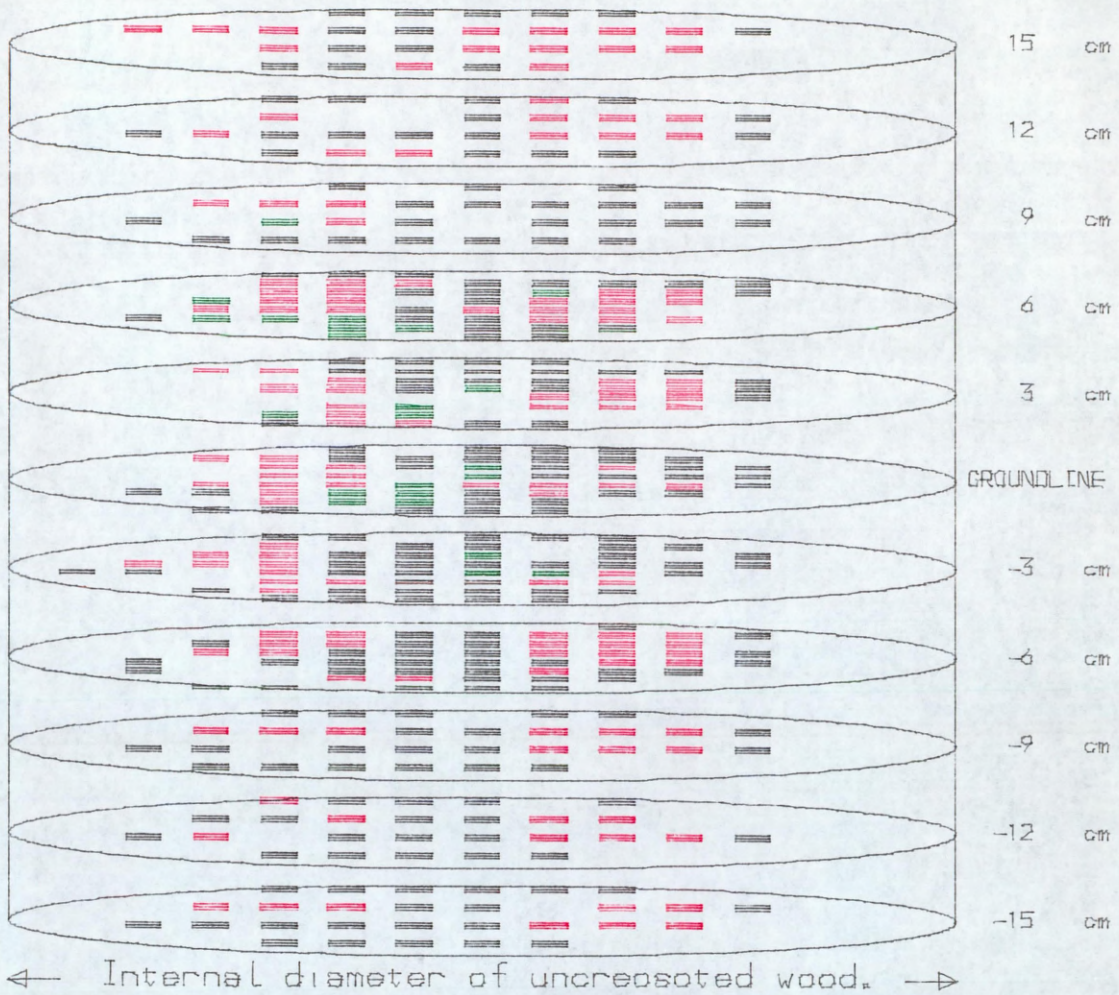
GREEN:- Trichoderma isolates.
RED :- Lentinus lepideus
BLACK:- Other resident pole organisms



Pole number: XC1 Sectioning date: 16/6/83
FYT pellet inoculation date: 11/5/82
Lentinus inoculation date: 15/6/81

KEY

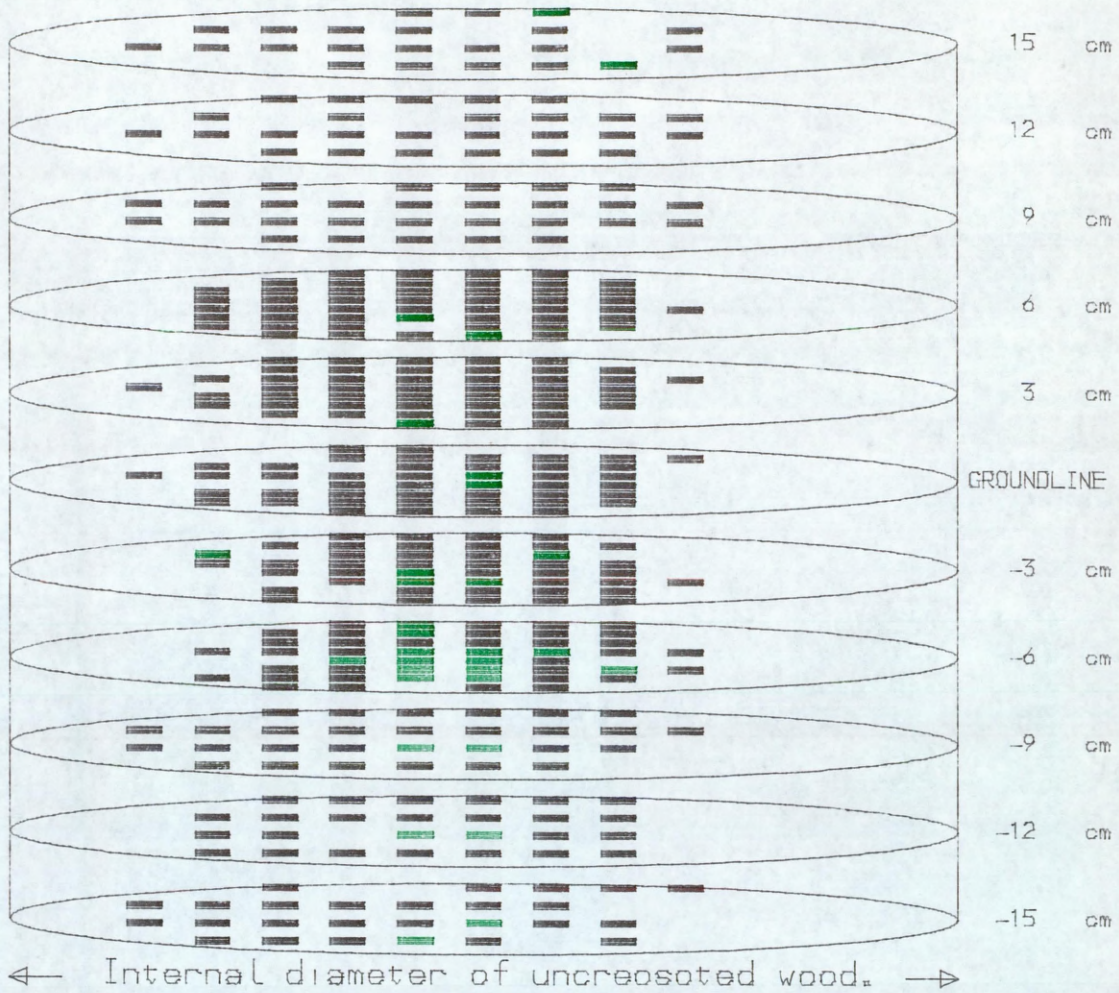
GREEN: - Trichoderma isolates.
RED : - Lentinus lepideus
BLACK: - Other resident pole organisms



Pole number: XF3 Sectioning date: 29/6/83
FYT pellet inoculation date: 11/5/82
Lentinus inoculation date: 15/6/81

KEY

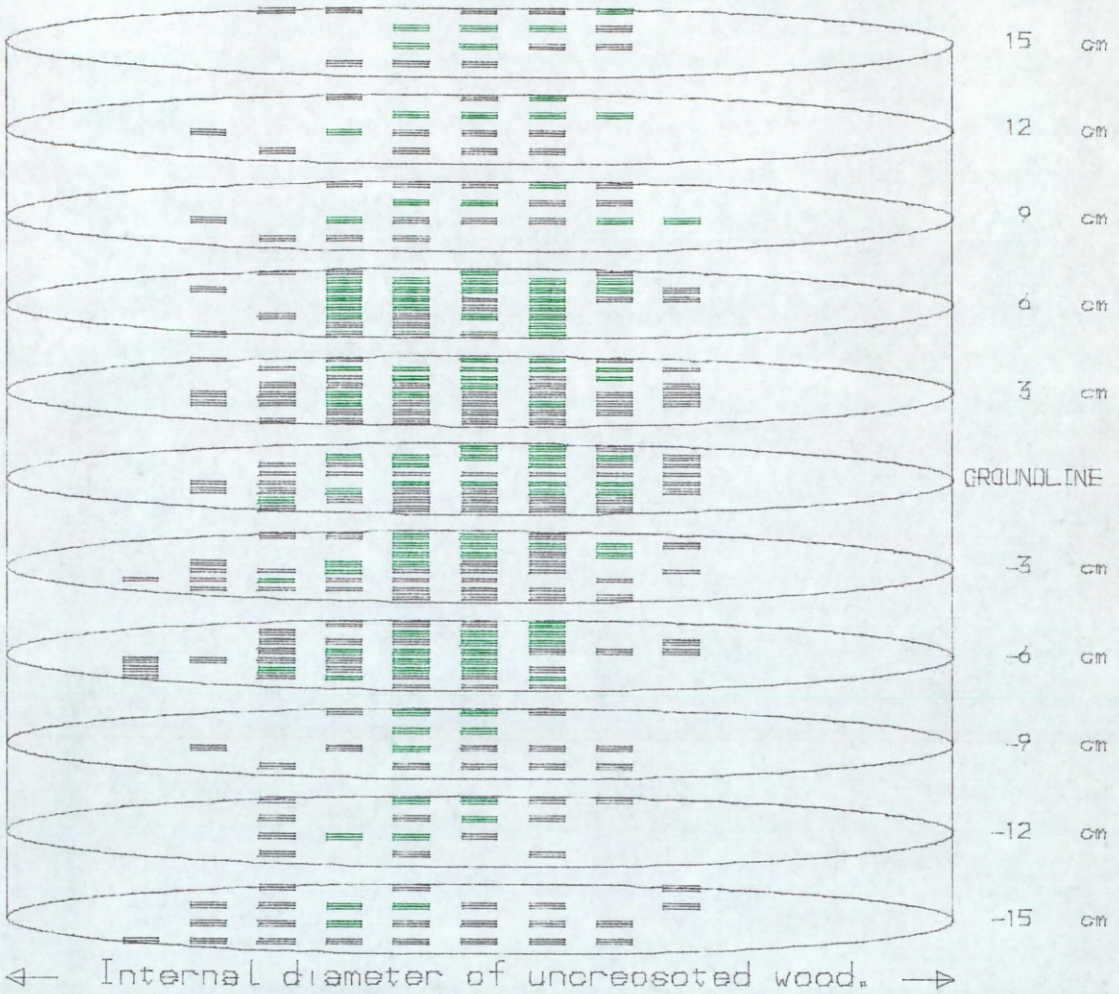
GREEN: - *Trichoderma* isolates.
RED : - *Lentinus lepideus*
BLACK: - Other resident pole organisms



Pole number: XF5 Sectioning date: 30/6/83
FYT pellet inoculation date: 11/5/82
Lentinus inoculation date: 15/6/81

KEY

GREEN:- Trichoderma isolates.
RED :- Lentinus lepideus
BLACK:- Other resident pole organisms



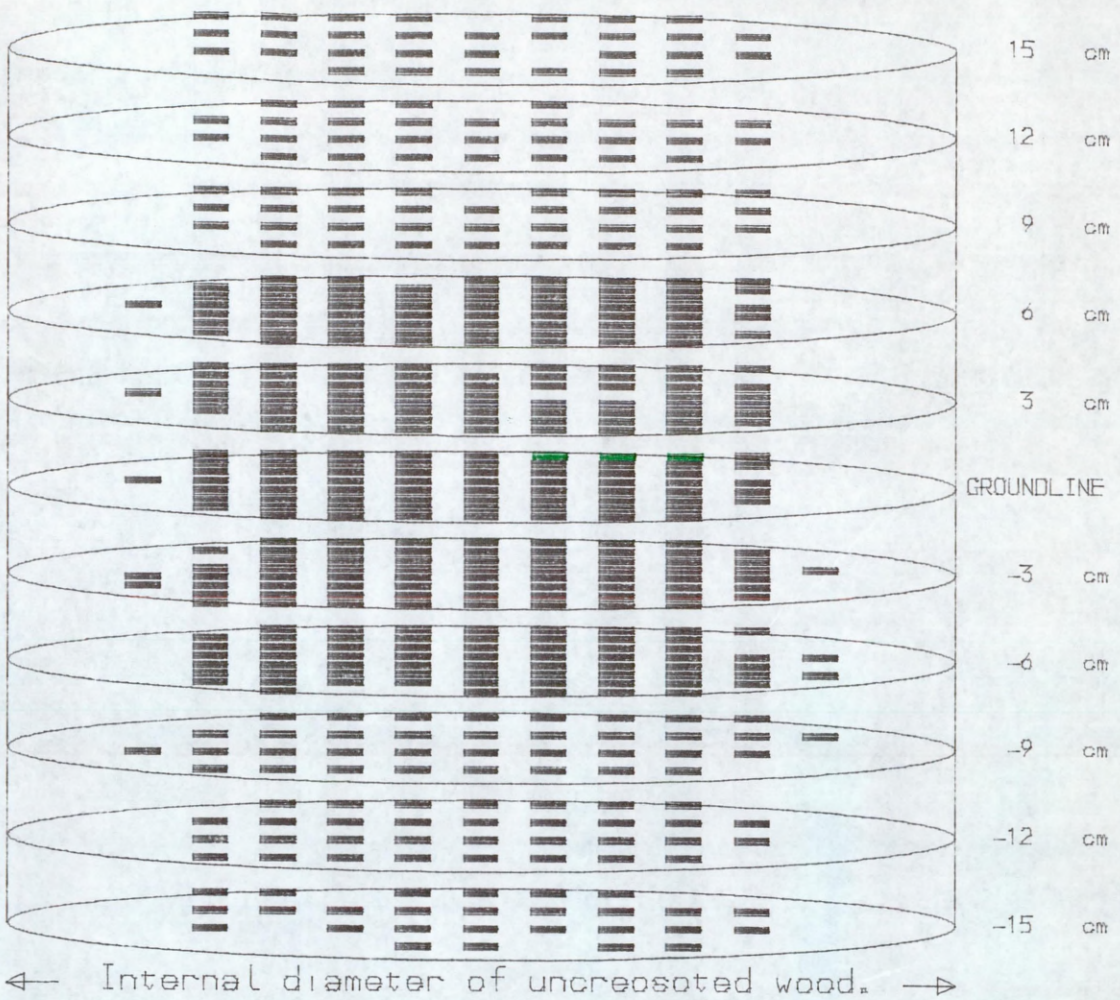
Pole number: XF2 Sectioning date: 1/7/83
FYT pellet inoculation date: 11/5/82
Lentinus inoculation date: 2/12/81

KEY

GREEN: - *Trichoderma* isolates.

RED : - *Lentinus lepideus*

BLACK: - Other resident pole organisms



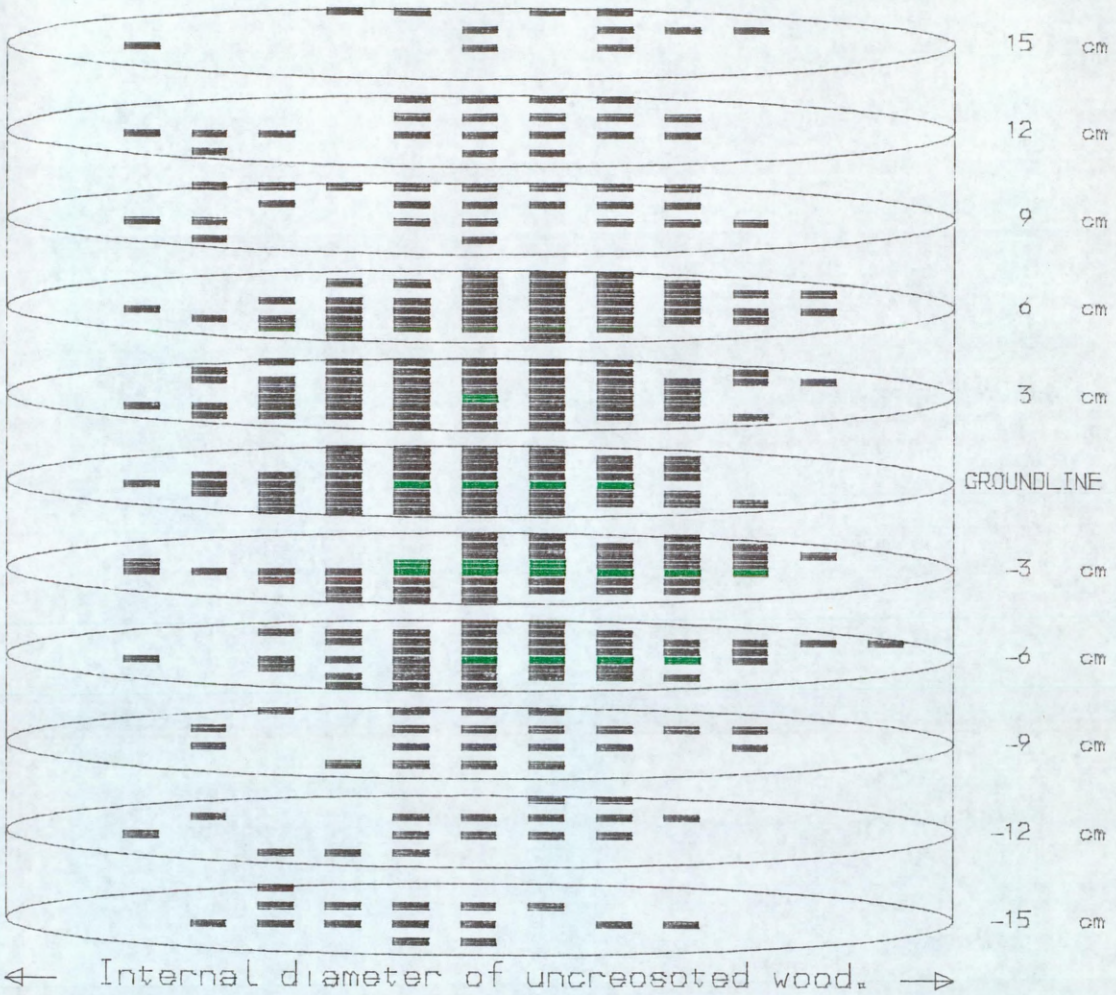
Pole number: XD1 Sectioning date: 28/6/83

FYT pellet inoculation date: 11/5/82

Lentinus inoculation date: 15/6/81

KEY

GREEN: - *Trichoderma* isolates.
RED : - *Lentinus lepideus*
BLACK: - Other resident pole organisms



Pole number: XC2 Sectioning date: 20/6/83
FYT pellet inoculation date: 11/5/82
Lentinus inoculation date: 2/12/81